Department of Food and Environmental Hygiene
Faculty of Veterinary Medicine
University of Helsinki
Finland

THE FOOD HYGIENIC QUALITY OF EUROPEAN RIVER LAMPREY (*LAMPETRA FLUVIATILIS* L.)

LAURI MERIVIRTA

ACADEMIC DISSERTATION

To be presented with the permission of the Faculty of Veterinary Medicine, University of Helsinki, for public examination in the Walter Auditorium, Agnes Sjöberginkatu 2, Helsinki, on 28 September, 2007 at 12 noon.

SUPERVISED BY:

Professor Hannu Korkeala

Department of Food and Environmental Hygiene

Faculty of Veterinary Medicine, University of Helsinki, Finland

and

Professor Johanna Björkroth

Department of Food and Environmental Hygiene

Faculty of Veterinary Medicine, University of Helsinki, Finland

REVIEWED BY:

Doz. Dr. Peggy Braun

Institute for Food Hygiene

Veterinary Faculty, University of Leipzig, Germany

Doz. Dr. Barbara Schalch

Institute for Hygiene and Technology of Food of Animal Origin

Veterinary Faculty, University Munich, Germany

OPPONENT:

Professor Anna-Maija Sjöberg

Department of Agrotechnology

Faculty of Agriculture and Forestry, University of Helsinki, Finland

ISBN 978-952-92-2389-3 (paperback)
ISBN 978-952-19-4062-7 (PDF)
Ai-Ri Offset Ky
Pori 2007

CONTENTS

ACKNOWLEDGEMENTS

This study was carried out at the Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine, University of Helsinki and at Porilab, City of Pori. The financial support of the Regional Council of Satakunta, the City Health Board of Pori and Finnish Cultural Foundation (Satakunta Cultural Foundation), as well as the Walter Ehrström Foundation are gratefully acknowledged.

I am deeply grateful to my supervisors Professor Hannu Korkeala, Head of the Department and Professor Johanna Björkroth for their supervision and evaluation of the publications of this study. Their encouragement and support during the project were overwhelming.

I would like to express my deepest gratitude for all my coauthors: Seija Berg, Meija Kivisaari, Dr. Joanna Koort, Dr. Miia Lindström, Jorma Nordlund and Prof. Kimmo Peltonen for contributing to the original publications.

I thank my coworkers at Porilab for their skills and competence in carrying out the various projects. I am also indebted to personnel at the Department of Food and Environmental Hygiene, Faculty of Veterinary Medicine of Helsinki University for their cooperation and friendship.

My special thanks belong to the personnel of all three lamprey processing plants. Without their smooth cooperation and assistance this study would have been impossible.

Finally, I owe my deepest thanks to my family, especially to my spouse, Tuire, for helping me to find the original idea and for her continuous support.

ABBREVIATIONS

AAS, atomic absorption spectrophotometry
AMV-RT, avian myeloblastosis virus-reverse transcriptase
AOAC, (formerly Association of Official Analytical Chemist), now ADAC International
APC, aerobic plate count
ATCC, American Type Culture Collection
CFU, colony-forming unit
DDT, dichlorodiphenyltrichloroethane
EC, Commission of the European Communities
EU, European Union
EYA, egg yolk
HCB, hexachlorobenzene
HCH, hexachlorocyclohexane
HELCOM, Helsinki Commission
HELCOM Countries, Denmark, Estonia, the European Community, Finland, Germany, Latvia, Lithuania, Poland, Russia and Sweden
ISO, International Organization for Standardization
LAB, lactic acid bacteria
MPN, most probable number
MRS, deMan, Rogosa, Sharpe
NCFA, Nordic Committee on Food Analysis
PCB, polychlorinated biphenyl
PCR, polymerase chain reaction
PFGE, pulsed-field gel electrophoresis
POP, persistent organic pollutants
REA, restriction endonuclease analysis
RFLP, restriction fragment length polymorphism
RH, relative humidity (%)
SSO, specific spoilage organisms
TEF, toxic equivalency factor
TNCL, transnonachlor
TPGY, tryptone-peptone-glucose-yeast extract broth
TSE, tryptose sulphite cycloserine
UPGMA, unweighted pair-group method using arithmetic averages
WHO, World Health Organization

ABSTRACT

The contaminants and microbiological quality of European river lamprey *Lampetra fluviatilis* L. were examined. The residues of mercury, cadmium, lead, hexachlorocyclohexanes (alpha-HCH, beta-HCH, lindane), hexachlorobenzene (HCB), heptachloroepoxide, dieldrin, chlordanes (oxychlordane, trans-chlordane, cis-chlordane, transnonachlor [TNCL]), DDT compounds (p,p'- DDE, p,p'- DDD, p,p'- DDT, o,p'- DDT), and 15 PCB congeners were determined in raw lampreys from several rivers in Finland and compared with respect to geographical areas. The residue concentrations were determined to evaluate the food safety of lamprey products. The results varied geographically. The levels og Hg and Cd residues were higher in lamprey caught in the Bothnian Bay area than in those from the Bothnian Sea. Pb was detected in only one sample. The mean ΣPCB, DDT, beta-HCH, HCB, dieldrin, oxychlordane and ΣDDT levels were significantly higher in samples from rivers flowing downstream to the Bothnian Sea in comparison to levels in the Bothnian Bay. There were no statistical differences between the areas, considering the concentrations of alpha-HCH, heptachloroepoxide, trans-chlordane, cis-chlordane and TNCL. All residue concentrations were well below the limits with regard to legislation.

The microbiological quality of charcoal-grilled lamprey in three lamprey processing plants was examined in products stored at 3 and 22 °C. On the day of production the mean aerobic plate counts (APC) in grilled lampreys were 2.29 log CFU/g in plant A and under the detection limit (2.0 log CFU/g) in plants B and C. At 22 °C the mean APCs of samples from plants A, B and C increased markedly within 4 days, and after 6 days were 8.56, 5.04 and 6.23 log CFU/g, respectively. The numbers of bacteria in charcoal-grilled river lamprey from plant A were higher than those from plants B and C. No significant increases in APCs were observed during storage at 3 °C for 24 days; the mean APC did not exceed 2.80 log CFU/g in samples from any plant.

Microbiological and sensory changes in vacuum-packed charcoal-grilled river lampreys from three lamprey processing plants were monitored as a function of time at 8 °C. The lampreys were examined every 7 days up to 8 weeks for APCs and LAB. The mean aerobic bacterial and LAB levels developed differently in samples from different plants. In

samples from plants A and C aerobic bacterial growth was detected after 2 weeks and growth of LAB after 3 and 5 weeks. In samples from plant B, aerobic bacterial growth was detected only after 6 weeks but no LAB growth during 8 weeks. Only 6 out of 15 lots attained an APC value of 7.0 log CFU/g during storage. The mean APC was higher after 4-8 weeks' storage than the mean levels of LAB. LAB were not the main bacterial group within the developing spoilage population. Twenty-seven LAB isolates were randomly picked from MRS agar and identified by ribotyping to species level. Twelve of the 27 isolates were identified as *Lactobacillus curvatus* subsp. *curvatus*, and two *Leuconostoc mesenteroides* and one *Weissella halotolerans* strain were also detected. Twelve isolates were not identified in the LAB database at the Department of Food and Environmental Hygiene, University of Helsinki, Finland, and were therefore subjected to 16S rRNA sequence analysis. The isolates selected to represent these 12 isolates possessed very high (99.9%) 16S rRNA gene sequence similarity with either *Staphylococcus warneri*- or *Staphylococcus pasteuri*-type strains. The sensory scores remained at the baseline levels after 8 weeks' storage. Microbial development in charcoal-grilled river lampreys is slow and the shelf life of the product is longer than that of some other heat-treated fishery products.

The food-borne pathogenic bacterial species *Clostridium botulinum* was studied in raw lampreys and *Clostridium perfringens*, *Listeria monocytogenes* and *Staphylococcus aureus* in the charcoal-grilled product. One out of 67 raw lampreys (1.5%) was positive for the botulinum neurotoxin type E gene as determined with PCR, with the estimated *C. botulinum* count being 100 spores per kg. Two type E strains were isolated from the positive sample and confirmed as different genotypes. Neither *C. perfringens* nor *L. monocytogenes* was detected, while *S. aureus* was found in two out of 300 samples (0.67%).

LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following original articles referred to in the text by the Roman numerals I to V:

I Merivirta, L., Nordlund, J. and Korkeala, H. 2001. Cadmium, mercury and lead content of river lamprey caught in Finnish rivers. Arch. Lebensmittelhyg. 52:69-71.

II Merivirta, L., Kivisaari, M., Berg, S., Peltonen, K., Björkroth, J. and Korkeala, H. 2006. Accumulation of PCBs and organochlorine pesticides in river-caught European river lamprey (*Lampetra fluviatilis)* in Finland. Bull. Environ. Cont. Tox. 76:497-504.

III Merivirta L., Björkroth, J. and Korkeala, H. 2003. Microbiology of charcoal-broiled European river lampreys (*Lampetra fluviatilis*) stored at 3 and 22°C. J. Food Prot. 66:2332-2335.

IV Merivirta, L., Koort, J.M.K., Kivisaari, M., Korkeala, H. and Björkroth, J. 2005. Developing microbial spoilage in vacuum-packaged charcoal-broiled European river lamprey (*Lampetra fluviatilis*). Int. J. Food Microbiol. 101:145-152.

V Merivirta, L., Lindström, M., Björkroth, J. and Korkeala, H. 2006. The prevalence of *Clostridium botulinum* in European river lamprey (*Lampetra fluviatilis*) in Finland. Int. J. Food Microbiol. 109:234-7.

1 INTRODUCTION

Charcoal-grilled European river lamprey *Lampetra fluviatilis* L. is a seasonal delicacy in Finland and throughout the Baltic Sea area. Fishing for river lamprey in Finland originated at least as far back as the 1500s and is well documented, lamprey being at that time a taxable item (Tuunainen et al., 1980). Despite the long history of the lamprey on the gourmet's menu, there is no information available on charred river lamprey as a food item.

Since lampreys live part of their lives in bottom sediments, it was important to investigate the residues of some heavy metals such as mercury, cadmium and lead. Lampreys have a fat concentration of approximately 14% and consume fish; thus it was also important to determine the amounts of polychlorinated biphenyls (PCBs) as well as dichlorodiphenyltrichloroethane (DDT) and other noxious organochlorine compounds.

Modern packaging techniques have enabled production of items with extended shelf lives. There is increasing interest among lamprey producers in vacuum-packing. The lactic acid bacteria (LAB) are often involved in the spoilage of vacuum-packed fish products (Truelstrup Hansen, 1995; Leroi et al., 1998; Lyhs et al., 1998, 1999). Therefore the microbial development in charred lampreys packed under vacuum was studied to ensure an adequate shelf life.

Traditionally the charcoal-grilled product is brought to the market without chilling and therefore it is essential to have knowledge of the microbiological and hygienic quality of lamprey. *Clostridium botulinum, Clostridium perfringen*s, *Listeria monocytogenes* and *Staphylococcus aureus* are bacterial species commonly known to cause food poisoning outbreaks involved with fish and fishery products (Autio et al., 2004). Recognition of the hazard presented by these foodborne pathogens in non chilled fish products was a particular reason for this study. The microbiological spoilage of raw lamprey is not included here because lamprey processing plants use only fresh lamprey. Raw lamprey are likewise not sold at fish markets.

2 REVIEW OF THE LITERATURE

2.1 Classification of lampreys

About 40 species of lampreys are found in the cool waters of both hemispheres. Their origin dates back at least 300 million years and lampreys are the most primitive extant vertebrates (Hardisty, 1983). The genus *Lampetra* including *L. fluviatilis* is a member of the subfamily *Petromyzontinae* in the family *Petromyzontidae*. This family is part of the order *Petromyzontiformes* and further of the superorder *Cephalaspidomorpha,* class *Cephalaspidomorphi*, superclass *Agnatha* and subphylum *Vertebrata* (Nelson, 1994). Members of the superclass *Agnatha* are normally considered as fish or fishlike and treated as fish in books. Lampreys are eel-like jawless vertebrates with a cartilaginous skeleton. They are devoid of a mineralized skeleton, although traces of globular calcified cartilage may occur in the endoskeleton.

The European river lamprey is widely distributed throughout the Baltic Sea, in coastal waters and rivers of the Mediterranean Sea and along the European Atlantic coast. Landlocked populations live in freshwater lakes in Finland, Norway, Russia and Scotland. There are three lamprey species in Finland (Tuunainen et al., 1980). The European river lamprey is common along the entire coast of Finland in the Baltic Sea. The European brook lamprey *Lampetra planeri* (Bloch), a non parasitic species, is found in freshwater throughout Finland. The sea lamprey *Petromyzon marinus* L. has been found only occasionally along the southern coast of Finland.

2.2 Life cycle of river lamprey

Some lamprey species live in freshwater throughout their lives but others such as the river lamprey are anadromous. They hatch and grow in rivers, migrate to the sea to mature and return to the rivers for spawning (Janvier, 1997). They are either parasitic or nonparasitic and both types may exist in individuals of the same species. The nonparasitic phase reproduces after metamorphosis without feeding. Parasitic lampreys attach to fishes by a

sucker equipped with numerous denticles, scrape their skin with horny teeth and rasplike tongue and feed on the bodily fluids.

The spawning period begins in the rivers in August and ends in May. Lampreys stop feeding at the beginning of migration (Ikonen et al., 1983) and they use the energy deposited in their fat. The mean fat concentration is 44% and 47% of dry weight in females and males, respectively (Laukkanen et al., 1979). The female deposits thousands of small yolk-free eggs in excavations in the sand of river bottoms. The spawning sites are usually situated downstream from rapids. Egg densities can be high and were estimated to be many thousands per m² (Tuikkala, 1971). After spawning the inner organs of lamprey degenerate disturbing the water balance, and the lampreys die. Lamprey eggs hatch into small larvae that are not predators. The larval lamprey, or ammocoete, has no sucker and poorly developed eyes. The gills of the larva are not enclosed in pouches and it feeds by trapping minute food particles with a strand of mucus produced by the pharynx. During the day the larvae hide in the bottom sediments of rivers. The density of larvae 1-3 years of age in the bottom sediment may increase to almost 100 individuals per m^2 (Kainua and Valtonen, 1980). The diet of larvae is composed of detritus, small insects, algae and small protozoa and bacteria (Järvenpää and Westman, 1986). The larval stage lasts 4-6 years and after a radical metamorphosis the lampreys migrate to sea. Downstream migration occurs mainly in spring during floods at night. In the sea lampreys begin feeding on fish species such as Baltic herring *Clupea harengus membras* L., sprat *Sprattus* (formerly *Clupea*) *sprattus sprattus* L. and Atlantic cod *Gadus morhua* L. (Tuunainen et al., 1980). After a 2-year growth period their length is approximately 25-30 cm and weight about 50 g. At that age they begin to migrate back to the rivers to reproduce. River lamprey do not exhibit marked homing behaviour.

2.3 Fishing and processing of river lamprey

Throughout the Baltic Sea area lampreys are caught for commercial purposes e.g. in Finland, Sweden and Estonia (Frimodt, 1995) and processed by artisanal food manufacturers. The fishing season, at the beginning of the spawning period, is normally from August to December. Generally the largest lampreys migrate earlier and the mean

weight of lampreys decreases during the autumn (Järvenpää and Westman, 1986). The adult lamprey weighs about 50 g. Fishing for lampreys has diminished during the last 25 years. The catch was about 2.7-3.0 million in the 1970s and in 1980 the estimated catch was 2.0-2.5 million individuals (Tuunainen et al., 1980). In 1997 the official yearly catch was 25 000 kg (Official Statistic of Finland, 1999). The catches for 2000, 2001, 2002 and 2003 numbered 1.8, 1.6, 1.3 and 0.55 million individuals, respectively (Raitaniemi et al., 2004).

The older device used for lamprey fishing was a wooden lamprey basket made of willow. Today most the commonly used gear are fyke nets (70% of the catch) or trap net (30% of the catch). Since migration occurs at night, the traps are set out in the evening and checked in the morning. In full moonlight the catches are known to be very poor and the traps are not set out. The catch is transported to processing plants immediately by truck or even by air.

The charcoal-grilling procedure used by producers is traditional, originating from earlier centuries. Charcoal-grilling is the most common commercial method for processing lampreys. The methods used to produce charred lamprey and the know-how are passed down from father to son. The product is normally delivered to the market without further processing. Lampreys are sold in booths and eaten as such or as a component of hors-d'oeuvre. Charcoal-grilled lampreys pickled with vinegar and spices and canned charred products can occasionally be found in the market as well.

The processing and handling of lampreys differ from that of other fishery products. In hot-smoking the fish is processed at temperatures of at least 70 °C and is therefore cooked in addition to being exposed to smoke (Lyhs, 2002). The temperature in addition to the bacteriostatic and bacteriocidal compounds of smoke eliminate or reduce the amount and number of microbial strains. In the cold-smoking process, not used for lampreys, the temperature is held under 28 °C and the normal microbial flora is not destroyed, including the pathogens. Charcoal-grilling occurs at higher temperatures than hot-smoking, the smoke being present in small amounts. In lamprey processing plants no raw materials or products other than lampreys are present, and thus cross-contamination may occur only from fresh lamprey or from the environment to the cooked product.

Manufacturers usually add 1 kg crystalline NaCl for every 50 kg of lampreys, although this may vary according to the consistency or the catchment area of the lamprey. Fresh lampreys are rubbed in the round with salt in a motorized apparatus resembling a concrete mixer. The mixing time in the running apparatus varies from 1 h to 1 h 30 min, depending on the size, colour and origin of the lampreys. After salting, the lampreys are rinsed at room temperature under running tap water for 5 min; the water used is controlled by municipal authorities. After a waiting period of approximately 12 h in a coldroom at 10 °C, the lampreys are grilled in ovens on charcoal made from burning alder. The lampreys are placed on a special grill on wheels and pushed into the oven. The temperatures measured in the oven of one producer varied during grilling between 116 and 187 °C (minimum 100 °C, maximum 292 °C), averaging 153 °C (Merivirta, 2006). The grilling time varied from 16 to 23 min. The temperature inside the lamprey increases in 2-3 min to 100 °C. The means minimum and maximum temperatures inside lampreys during grilling are 90 °C and 114 °C, respectively. On the other hand, the temperature in the oven and also the charring time may vary depending on the grillmaster in charge. Lampreys are normally turned once on the grid during grilling. Part of the lot may be reheated if not satisfactorily grilled. Chilling occurs at room temperature and the product is then usually delivered to the market without further processing. Some producers occasionally prepare pickled products with vinegar and spices. There is increasing interest in vacuum-packing of the product, but the influence of the procedure on lampreys is unknown.

2.4 Residues in fish and fishery products

2.4.1 Mercury, cadmium and lead residues

Marine Hg originates from soil mainly by weathering and leaching. In addition burning of oil, charcoal and waste and some industrial processes generate Hg emissions. The noxious effects of Hg on animals and humans are caused by the derivatives mono- or dimethylmercury. The toxic element Cd is emitted from the metal industry, power plants, waste burning and also from certain fertilizers (deBoo, 1990). In the industrialized countries Pb has been a problem, mainly caused by petrol containing lead and the

accumulator industry. The total atmospheric emissions of Hg, Cd and Pb in the countries surrounding the Baltic Sea were in year 2003 61, 116 and 3271 tonnes, respectively [Helsinki Comission (HELCOM), 2007]. These metals are persistent, with biological half-lives measured years, and accumulate in tissues. Heavy-metal concentrations in the Baltic Sea are many times higher than in the North Atlantic and have not decreased since the 1990s. In general the 2003 results Hg, Cd and Pb concentrations in Baltic Sea surface and deep waters revealed no pronounced changes compared with previous years (HELCOM, 2007).

Several studies in the Baltic Sea area have monitored the levels of trace metals (Hg, Cd and Pb) in fish and fishery products (Table 1). The concentration of Hg in lampreys varied between 330 and 480 μg kg^{-1} (Kukko and Turunen, 1973). The levels show a discrepancy, depending on the aquatic environment. The Hg concentrations found in fish are higher in the southern and northern parts of the Baltic Sea than in the central regions and Pb levels are higher in the southern Baltic than in the Gulf of Bothnia. Several studies have examined fish samples from the southern Baltic. Falandysz (1985b) found the concentrations of Pb >120 μg kg^{-1} in flatfish [turbot *Psetta maxima* L. (formerly *Scophtalmus maximus* L., plaice *Pleuronectes platessa* L. and European flounder *Platichtys flesus* L.]. In cod the concentrations were 5 and 86 μg kg^{-1} for Cd and Pb, respectively (Falandysz, 1986b). The residues of Hg, Cd and Pb in Patagonian squid *Loligo patagonica* Smith from the southern Baltic Sea were 12, 320 and 1000 μg kg^{-1}, respectively (Falandysz, 1989). Cd and Pb were found in cod, Baltic herring, sprat and some other fish in amounts presented in Table 1 (Szefer and Falandysz, 1985).

Table 1. Fresh weight (µg kg^{-1}) concentrations of mercury, cadmium and lead in fish caught from the Baltic Sea.

Fish species	Location	Hg	Cd	Pb	Reference
Baltic herring	Baltic Sea	<5-110	2-9	<10-30	Venäläinen et al., 2004
Burbot	Baltic Sea	200-350	<1	<10	Venäläinen et al., 2004
Cod	Southern Baltic Sea	ND	2	60	Szefer and Falandysz, 1985
Cod	Southern Baltic Sea	ND	5	86	Falandysz, 1986b
Eel	Southern Baltic Sea	ND	3	50	Szefer and Falandysz, 1985
Flounder	Southern Baltic Sea	ND	13	120	Falandysz, 1985b
Flounder	Baltic Sea	40-50	1	<10	Venäläinen et al., 2004
Flounder	Southern Baltic Sea	ND	4	90	Szefer and Falandysz, 1985
Fourbearded rockling	Southern Baltic Sea	ND	2	40	Szefer and Falandysz, 1985
Garfish	Southern Baltic Sea	ND	4	70	Szefer and Falandysz, 1985
Greater sand eel	Southern Baltic Sea	ND	35	150	Szefer and Falandysz, 1985
Herring	Southern Baltic Sea	ND	13	90	Szefer and Falandysz, 1985
Lamprey	Gulf of Bothnia	330-480	ND	ND	Kukko and Turunen, 1973
Perch	Baltic Sea	80-1350	<1-2	<10-20	Venäläinen et al., 2004
Pike	Baltic Sea	150-850	<1-2	<10-20	Venäläinen et al., 2004
Pikeperch	Baltic Sea	68-180	1	<10	Venäläinen et al., 2004
Plaice	Southern Baltic Sea	ND	9	170	Falandysz, 1985b
Salmon	Baltic Sea	ND	3	10	Szefer and Falandysz, 1985
Salmon	Baltic Sea	50-100	10	<10-10	Venäläinen et al., 2004
Sprat	Baltic Sea	20-30	6-38	<10	Venäläinen et al., 2004
Sprat	Southern Baltic Sea	ND	19	90	Szefer and Falandysz, 1985
Squid	Southern Baltic Sea	12	320	1000	Falandysz, 1989
Stickleback	Southern Baltic Sea	ND	40	850	Szefer and Falandysz, 1985
Turbot	Southern Baltic Sea	ND	12	160	Falandysz, 1985b
Whitefish	Baltic Sea	20-30	<1-3	<10-20	Venäläinen et al., 2004
Whiting	Southern Baltic Sea	ND	5	50	Szefer and Falandysz, 1985

ND, not determined

The concentrations of Hg and Cd found in fish from the Gulf of Finland and Gulf of Bothnia vary depending on the species. The highest amounts of Hg have been found in predatory fish such as northern pike *Esox lucius* L. (850 µg kg^{-1}) and European perch *Perca fluviatilis* L. (1350 µg kg^{-1}). The mean amount of Hg was higher in Atlantic salmon *Salmo salar* L. (70 µg kg^{-1}) than in Baltic herring (30 µg kg^{-1}). The levels of Cd were higher in salmon (11 µg kg^{-1}) and Baltic herring (9 µg kg^{-1}) than in common whitefish *Coregonus lavaretus* L. (2 µg kg^{-1}), pikeperch *Sander* (formerly *Stizostedion*) *lucioperca* L. (1 µg kg^{-1}) and flounder (1 µg kg^{-1}); lamprey was not included (Venäläinen et al., 2004). Pb was under or near the detection limit of 10 µg kg^{-1}. Since it is essential to protect public health and to maintain contaminants at levels that do not cause health concerns, the European Union (EU) has set maximum levels for certain contaminants in foodstuffs including Hg (500 µg kg^{-1}), Cd (50 µg kg^{-1}) and Pb (200 µg kg^{-1}) in fish [Commission of the European Communities (EC), 2005].

2.4.2 PCBs and pesticide residues

The PCBs are a group of toxic, environmentally persistent, and lipophilic organic compounds that have been used worldwide since 1929. The applications have been mainly as oils in transformers and capacitors, but also as additives in inks, heat-exchange fluids and copy papers. Most developed countries and the EU have severely restricted the use of PCB-containing products since 1976. In Finland the use of PCBs was prohibited in 1990 and transformers containing PCBs were removed by 1994. The PCBs are composed of various numbers of chlorine atoms in the biphenyl moiety. About 150 congeners among the 209 possible are reported to be present in the environment. In 1998 the World Health Organization (WHO) driven consensus for the toxic properties of 12 selected PCBs — the so-called toxic equivalency factors (TEFs) — was launched (van den Berg et al., 1998). However, the remaining congeners may have toxicological effects in the environment and also in humans. Giesy and Kannan (1998) reported dioxin-type toxic effects caused not only by congeners with dioxinlike structure but also those with different substitution pattern of chlorine atoms. Recently, various methods for estimating the total nondioxinlike PCB mass in fish tissue samples were examined (Connor et al., 2005).

DDT was used during World War II and later as an insecticide. DDT and its metabolites DDD and DDE are environmentally persistent and the half-life is 16 years. DTT is also spread through the atmosphere. It is still used against malaria, mainly in the developing countries. Dieldrin was used to control agricultural pests of maize, potato and cotton. Heptachlor has been known since 1946 and has been used to control insects and malaria. Chlordane and γ-hexachlorocyclohexanes (HCHs) such as lindane and its α- and β-isomers have been used as insecticides in horticulture and are probably still used to control termites in some countries. They have also been used in veterinary medicine against scabies. Hexachlorobenzene (HCB) has been used as a fungicide, solvent and impregnator since 1945.

A number of countries have banned several pesticides during recent decades. In Finland the use of dieldrin and DDT was prohibited in 1972, lindane in 1987, and heptachloroepoxide, HCB and chlordane in 1996. Depending on the half-lives of these compounds, the levels of organohalogen residues will probably be further reduced in the long run (Mackenzie et al., 2004).

PCBs and organochlorine pesticide residues are very commonly found in fish, fishery products, and marine biota in which they are often reported as contaminants. Falandysz (1985a) reported that the levels of organochlorine pesticide residues in sprat from the southern Baltic were 2-3 times higher than those found in the same area in 1981 but that the PCB levels differed only slightly. In the same period the respective residues in herring in the same region (Falandysz, 1986a) were 2-3 times higher than those observed 2 years earlier; only the PCBs remained at the previous level. The residues in flatfish from the southern Baltic were 71, 66 and 94 μg kg^{-1} wet weight for PCB, 16, 18 and 40 μg kg^{-1} for ΣDDT and 0.7, 0.88 and 1.1 μg kg^{-1} for HCB in turbot, plaice and flounder, respectively (Falandysz, 1985c). In cod the mean levels found for muscle tissue were 55, 10 and 0.65 μg kg^{-1} wet weight for PCBs, ΣDDT and HCB, respectively (Falandysz, 1986c). In a study on smoked European eel *Anguilla anguilla* L. originating from different countries (Denmark, Germany, Sweden and Poland) around the Baltic Sea, the concentration of alpha-HCH, beta-HCH, lindane, HCB, dieldrin and DDT compounds varied 16-76, ND (not detected) -34, 11-42, 8-360, 30-89 and 8-348 μg kg^{-1} fat (Karl and Lehmann, 1993).

The ΣPCB concentrations in fish muscle showed significant declining trends in the biotic matrices investigated as a result of measures taken to reduce discharges of PCBs to the environment. The concentrations are still significantly higher in the Baltic Proper and in the southern Bothnian Sea than in the Kattegatt and the Skagerak (HELCOM, 2007). The decreasing rates in herring muscle vary between 4.1% and 9.7% per year. The concentrations of PCBs and lindane in herring have both decreased significantly in the Baltic Sea, probably due to the effect on emissions of stricter regulations and bans in the HELCOM countries (Denmark, Estonia, the European Community, Finland, Germany, Latvia, Lithuania, Poland, Russia and Sweden).

The levels of HCH, DDT, HCB and PCBs found in herring in the Bothnian Bay and Gulf of Gdansk were similar to and generally lower than those in the Bothnian Sea for all chemicals except DDT, whose concentration was highest in the Gulf of Gdansk. For the perch samples the industrialized location had markedly higher concentrations of HCB and PCBs than the other locations (Strandberg et al., 1998a). This species also identifies the Gulf of Gdansk as the most DDT-contaminated site among the three areas mentioned above.

The concentrations of ΣPCB in perch in Latvian coastal area were similar at all study locations (0.7-1.4 ppm on a lipid weight [l.w.] basis), although a site near the city of Riga indicated the presence of a local PCB contamination source (Olsson et al., 1999). The content of persistent organic pollutants (POPs) in perch and flounder from the coastal waters of Estonia were lower than the standards set by the WHO (Roots, 2001). The levels of PCBs in Baltic herring were lower in catches from Gulf the of Finland than in those from the Gulf of Bothnia, and no decreasing trend was found in concentrations between 1993-94 and 1999 (Kiviranta et al., 2003). In 1999 the ΣPCB in small and large herring were 69 and 94 μg kg^{-1} in the Gulf of Finland and 71 and 180 μg kg^{-1} on a fat weight basis in the Gulf of Bothnia, respectively. The amounts of PCBs in salmon from the Bothnian Sea area varied 242-289 μg kg^{-1} and in Baltic herring between 8.61 and 303 μg kg^{-1} wet weight, depending on the age of the fish (Hallikainen et al., 2004). The concentrations of ΣPCB from the Gulf of Bothnia in perch, pike and burbot *Lota lota* L. varied 12.2-97.0 μg kg^{-1}, 14.2-26.4 μg kg^{-1} and 1.15-2.35 μg kg^{-1}, respectively.

Nakari et al. (2002, 2004) monitored the residues of PCBs, DDT, and some pesticides in Baltic herring and pike from the Bothnian Bay and Bothnian Sea during 1997-1999 and 2000-2002. They showed that the concentrations of the analytes were higher in Baltic herring than in pike. Nakari et al. (2004) observed that the concentrations of beta-HCH, alpha-HCH, lindane, HCB, transnonachlor (TNCL) and ΣDDT in Baltic herring from the Bothnian Sea and Bothnian Bay 0.61 and 0.18, 1.3 and 0.11, 5.9 and 0.093, 3.5 and 0.56, 3.1 and 0.76, and 29.8 and 5.98 μg kg^{-1} wet weight, respectively. The compounds measured are lipid-soluble and their concentrations were higher in fish species with higher fat content.

Few studies have focused on the various residues in lampreys. Specific accumulation of PCBs and their congeners in edible fish, including lampreys, in the southern part of the Baltic Sea were reported (Falandysz et al., 2002), and the level of PCBs in lampreys varied between 110 and 230 μg kg^{-1}. Chlorinated cyclodiene pesticide residues were found in lampreys in the southern Baltic (Falandysz et al., 2001). The concentrations of cis-chlordane, trans-chlordane, TNCL, oxychlordane, heptachloroepoxide and dieldrin were 1.8, 0.31, 2.1, 0.83, 0.03 and 3.8 μg kg^{-1} fresh weight, respectively. The POP residues in contaminated bottom sediment of rivers accumulate in lamprey larvae (Soimasuo et al., 2004). Although the larvae remain at least 4 years in the sediment, this impact is minor because the main growth occurs during the sea period when lampreys increase approximately 100 times in size.

The concentrations of PCBs and organochlorine pesticides are increasing in the food chain. This property of the compounds causes severe problems in piscivorous animals such as seals and sea birds, especially in their reproduction (Hario et al., 2000; 2004; Routti et al., 2005). As far as human exposure is concerned, one of the main sources of PCBs and other organochlorine compounds is fish and fishery products (Asplund et al., 1994). Organochlorine compounds in fish and bottom sediments are widely used as bioindicators to assess the degree of environmental contamination with persistent pollutants and especially to monitor the quality of the aquatic environment (Strandberg et al., 1998a, b, 2000; Falandysz et al., 2001).

The EU has set maximum levels for certain contaminants in foodstuffs, including dioxinlike PCBs in fish (EC, 2001). In addition there is a temporary derogation that Finland and Sweden are authorized to place on their market Baltic salmon, Baltic herring, river lamprey, brown trout *Salmo trutta* L., Arctic char *Salvelinus alpinus* L. and roe of vendace *Coregonus albula* L., originating from the Baltic region, which is intended for consumption in their territory with levels of dioxins and/or levels of the sum of dioxins and dioxinlike PCBs higher than those set in regulation (EC, 2006). The national authorities in Finland (Anonymous, 2003) have set action limits for some residues in fish: 20 µg kg^{-1} for lindane, 200 µg kg^{-1} for HCB, 100 µg kg^{-1} for heptachloroepoxide, 500 µg kg^{-1} for ΣDDT, and 2000 µg kg^{-1} for ΣPCB.

2.5 Microbial spoilage in heat-treated fishery products

2.5.1 Aerobic plate counts in association with sensory changes

Microbial spoilage is a result of the production of off-odours and -flavours caused by bacterial activity. Since only part of the total bacterial population causes sensory spoilage changes, these changes are not necessarily correlated with the total number of microbes. The spoilage-causing microbes in the microbial population present in spoiling food (Gram et al., 2002) are referred to as specific spoilage organisms (SSOs). The future aim is to develop methods for detecting SSOs in association with specified food types to substitute and complement nonspecific aerobic plate counts (APC). The APC method in fish products has been used as an indicator of the hygienic quality of the product. When the APC is used as an indicator for spoilage in fish products, the number must be > 6.0 log colony-forming units (CFU)/g before the sensory changes are manifested.

In 79% of samples of different hot-smoked fish, no sensory changes were present when the bacterial count was under 6.0 log CFU/g, but changes were reported in 90% of samples exceeding 6.0 log CFU/g (Kleickmann and Schellhaas, 1979). The APC and sensory quality were studied in samples of hot-smoked Atlantic halibut *Hippoglossus hippoglossus* L., Baltic herring and eel from nine commercial smokehouses (Karnop, 1980). In 3.5% of

halibut samples the APC values were below 2.0 log CFU/g, 74% in the range 3.0-6.0 log CFU/g, and 3.5% between 6.0 and 7.0 log CFU/g. Only in 4% of herring samples were the APCs between 2.0 and 3.0 log CFU/g. In all eel samples the APC was under 2.0 log CFU/g. The sensory quality was excellent in 68% of halibut samples and excellent or good in 53% and in 75% of herring and eel samples, respectively.

The chilling procedure and storage temperature are crucial for delaying bacterial development in fishery products. The APC in charred Baltic herring was after processing an average of 2.75 log CFU/g and increased up to 4.93 log CFU/g during a storage time of 96 hours at 4 °C (Korkeala and Pakkala, 1988). After storage at 20 °C the APC in the same product was an average of 9.23 log CFU/g. The values in hot-smoked Baltic herring were lower (8.01 log CFU/g). In vacuum-packed hot-smoked trout filets stored at 8 °C the APC was 6.0 log CFU/g after 7 days and stored at 4 °C after 13 days. Sensory changes were already present after 7 days in 25 samples out of 80 in products stored at 8 °C but after 16 days in six samples stored at 4 °C. Only 37% of samples out of 125 containing APCs > 6.0 log CFU/g showed sensory changes (von Zorn et al., 1993).

In a survey to determine the microbiological levels of cold-smoked and hot-smoked ready-to-eat fish, the initial APC values were under 5.0 log CFU/g in 77% out of 100 samples and less than 3.0 log CFU/g in 39% (Dodds et al., 1992). After 30 days at 4 °C, 56% of the APC values in samples were greater than 7.0 log CFU/g. The sensory evaluation was not included in this study. The APCs in mackerel, hot-smoked at a core temperature not exceeding 60 °C, in a plant of very high sanitary standard, was very low just after smoking, cooling, and packaging, ranging from 1.0 to 1.5 log CFU/g. The sensory quality of mild hot-smoked mackerel produced from high-quality raw material was stable for 14 days at 2 °C and at least 8 days at 6 °C (Sikorski and Kolodziejska, 2002).

In tuna smoked until the core temperature reached 50 °C, the APC counts were 6.1 log CFU/g immediately after smoking and after 10 weeks' storage at 5 °C in vacuum packages, while in pooled samples they attained levels of 9.0 log CFU/g with acceptable organoleptic properties (Paleari et al., 1989). This result contrasts with that of other studies showing an association between APC and spoilage. There are no results available on APC or sensory changes during storage of charcoal-grilled lampreys.

2.5.2 Lactic acid bacteria

The LAB are divided into several genera with a number of common physiological and metabolic characteristics. They are Gram-positive, rods (*Lactobacillus* and *Carnobacterium*) or cocci (*Aerococcus, Enterococcus, Lactococcus, Vagococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus* and *Tetragenococcus*), anaerobic, microaerophilic or airtolerant. The genus *Weissella* comprises both rods and cocci (Collins et al., 1993). The LAB produce lactic acid as the sole product or in addition to such metabolites as ethanol, acetic acid and CO_2 in glucose fermentation. Some LAB also exhibit proteolytic activity, e.g. some strains of lactococci used in the dairy industry. Taxonomically the principal LAB are *Aerococcus, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* (Axelsson, 2004). The LAB comprise mostly nonpathogenic bacteria with the exception of some species in the genera *Enterococcus* and *Streptococcus*. Recent advances in research on bacteria including LAB have been based on genetic and molecular biological applications. The identification of LAB strains in food products with molecular tools constitutes state-of-the-art methodology (Björkroth and Korkeala, 1996a, 1996b, 1997; Björkroth et al., 1998, 2000; Lyhs et al., 1999) that has increased the knowledge of their metabolism, taxonomy and interaction with other microbes.

The LAB have been documented as part of the microbiota in marine environments and several species are found in fish digestive tracts. Ringø et al. (2000) described carnobacteria associated with the digestive tract of Atlantic salmon. Eleven *Carnobacterium* strains were identified from various intestinal regions of Atlantic salmon, Arctic char, Atlantic cod and wolfish *Anarchichas* (Ringø et al., 2001). The following genera are found in the digestive tracts of fish: *Lactobacillus, Carnobacterium, Aerococcus, Enterococcus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus* and *Vagococcus*. Several other investigations have shown the presence of LAB in fish (Ringø, 2004).

The LAB often form the main bacterial group in vacuum-packed, slightly preserved fishery products stored at chilled temperatures. Some genera are involved in fermentation, others in spoilage and in many cases their influence is obscure.

A number of LAB species have been found in fermented fish products: *Lactococcus lactis* and *Lactobacillus brevis* in jeot-gal, a Korean fish product (Lee et al., 2000), *Lactobacillus acidipiscis* and *Weissella thailandensis* in Thai-style fish (Tanasupawat et al., 2000) and *Lactobacillus pentosus*, *Lactobacillus plantarum* and *Pediococcus pentosaceus* in a Thai low-salt fermented product (Paludan-Müller et al., 1999).

In spoiled fish products there is often a mixture of microbes at the time the product becomes objectionable to the senses. In vacuum-packed cold-smoked salmon three different types of bacteria were present, dominated by LAB, LAB and *Enterobacteriaceae* or LAB and *Photobacterium phosphoreum* (Truelstrup Hansen, 1995). In spoiled vacuum-packed, cold-smoked fish product several LAB genera are involved including *Lactobacillus*, *Leuconostoc*, *Lactococcus* and *Carnobacterium* (Gancel et al., 1997; Leroi et al., 1998; Paludan-Müller et al., 1998). Lyhs et al. (1999) found *Leuconostoc mesenteroides* subsp. *mesenteroides, Leuconostoc citreum, Lactobacillus sakei* and *Lactobacillus curvatus* to be the main species associated with spoilage of vacuum-packed, cold-smoked rainbow trout *Oncorynchus mykiss* (Walbaum). *Lactobacillus alimentarius* was the specific spoilage organism detected in spoiled marinated herring samples (Lyhs et al., 2001). High LAB levels (7.0-8.0 log CFU/g) are present several weeks in vacuum-packed, cold-smoked salmon before the product becomes objectionable to the senses (Truelstrup Hansen, 1995), therefore 'the total count of bacteria' only is not useful as a spoilage indicator (Gram and Huss, 1996).

LAB have been observed in many different types of fish products with or without spoilage, including cold-smoked herring (Magnússon and Traustadóttir, 1982) and vacuum-packaged, cold-smoked salmon and rainbow trout (Shimasaki et al., 1994; Civera et al., 1995; Truelstrup Hansen, 1995; Lyhs et al., 1998). *Carnobacterium maltaromaticum* predominated in 97 of the 155 LAB isolates from cold-smoked salmon (Leroi et al., 1998). *Carnobacterium piscicola* was the dominant LAB in spoiled cold-smoked, vacuum-packed salmon (Paludan-Müller et al., 1998). In samples of different hot-smoked fish, LAB counts

over 5.0 log CFU/g were present in 26.2% with and in 12.3% without sensory changes (Kleickmann and Schellhaas, 1979). In freshly hot-smoked halibut 75% of the microbiota were identified as LAB (Karnop, 1980). LAB increased in vacuum-packed smoked herring at the expense of other genera, being 76% after 9 weeks' storage, with the APC number exceeding the maximum 8.0 log CFU/g after 7 weeks (Magnússon and Traustadóttir, 1982). Enterococci were present in charred Baltic herring in amounts up to 7.16 log CFU/g in samples stored at 20 °C for 96 hours (Korkeala and Pakkala, 1988). In hot-smoked tuna the LAB counts were immediately after smoking 2.9 log CFU/g. After 10 weeks' storage at 5 °C in vacuum packages, the LAB counts in pooled samples attained 9.0 log CFU/g (Paleari et al., 1989). Jeppesen and Huss (1993) reported that all isolated LAB from samples of vacuum-packed minced herring were *Leuconostoc* spp. LAB were present in all samples of smoked vacuum-packed trout stored at 4 ºC, while 25% of samples contained more than 6.0 log CFU/g (von Zorn et al., 1993). No results are available on LAB or sensory changes occurring during storage of charcoal-grilled lampreys.

2.6 Bacteria related to safety of fish and fishery products

Pathogens in fish and fishery products can be of aquatic or other environmental origin or from human and animal reservoirs. Some are naturally present as the initial microbiota of fish and others as a result of cross-contamination and postprocess contamination.

2.6.1 *Clostridium botulinum* and *Clostridium perfringens*

Clostridium botulinum and *C. perfringens* are species of the genus *Clostridium* (family *Clostridiaceae*, order *Clostridiales*). *Clostridium botulinum* and *C. perfringens* are Gram-positive, anaerobic, rod-shaped and spore-forming bacteria. *Clostridium botulinum* is able to produce neurotoxins.

Clostridium botulinum strains are divided into four groups (I-IV), based on their metabolism and pathogenesis; groups I and II include the human pathogenic *C. botulinum* strains. Group I consists of proteolytic strains producing types A, B and F toxins, and

group II of nonproteolytic strains producing types B, E and F toxins (Smith and Sugiyama, 1988; Lindström and Korkeala, 2006). Consuming a small amount, as little as 30 ng, of neurotoxin is sufficient to cause severe illness, even death in humans (Novak et al., 2005). The minimum growth temperature for group II is 3.0 °C (Hauschild, 1989). Current molecular methods for detection and identification, such as polymerase chain reaction (PCR)-based tools and pulsed-field gel electrophoresis (PFGE) have enhanced the investigation of outbreaks caused by *C. botulinum* (Lindström et al., 2001, 2004).

Clostridium botulinum group II is commonly found in aquatic environments, especially in bottom sediments, and in the intestinal tracts and surfaces of fish. The nonproteolytic group II has predominated in surveys in the Baltic Sea area, and a particularly high prevalence of type E was reported in bottom sediments (Johannsen, 1962, 1963; Huss, 1980, Hielm et al., 1998a, Hyytiä et al., 1998). Furthermore, several surveys showed variable prevalences of *C. botulinum* type E in raw fish (Cann et al., 1965; Huss and Pedersen, 1979; Hielm et al., 1998b). *Clostridium botulinum* was surprisingly not present in 100 samples of ready-to-eat fish sold at retail level in the Toronto region, Canada (Dodds et al., 1992). Hyytiä et al. (1998) reported contamination levels of 10-40% in raw fish and fish intestines, the highest prevalence being in Baltic herring, and 4-14% in fish roe in Finland. Of Finnish hot-smoked fish products, including a lamprey sample, 5% contained spores, indicating that *C. botulinum* may survive the heat treatments employed by the food industry (Hyytiä et al., 1998) or caused by postprocessing contamination. Raw fish from Germany contained 30% type E spores (Hyytiä-Trees et al., 1999).

Thermal inactivation of nonproteolytic *C. botulinum* type E spores was examined (Lindström et al., 2003) and the decimal reduction time in whitefish medium was 7.1 min at 90 °C. This indicates that *C. botulinum* in fish products presents a health risk for humans if the heating process is not sufficient. Vacuum-packing and insufficient storage temperatures combined with long storage time add to the risk significantly.

A number of outbreaks and case reports involving *C. botulinum* type E are described: smoked whitefish (Anonymous, 1964) and smoked trout (Anonymous, 1968), vacuum-packed hot-smoked whitefish (Korkeala et al., 1998; Lindström et al., 2006), fermented salmon roe (Dawar et al., 2002), fish products (Boyer et al., 2001; Lindström et al., 2004)

and airtight-sealed smoked salmon (Dressler, 2005). There was a suspected association between consumption of smoked vacuum-packed whitefish and an outbreak caused by botulinum toxin type E (Lindström et al., 2006).

The optimal growth temperature for *C. perfringens* is 43-45 °C, although it can also grow at temperatures between 15 and 50 °C (Labbe and Juneja, 2002). The spores are resistant to heat and survive cooking. During sporulation it produces *C. perfringens* enterotoxin (CPE), which is the causative factor of gastroenteritis (McClane et al., 1988). *Clostridium perfringens* was isolated in 37 faecal samples (38.9%) from cod, and all isolates were *C. perfringens* toxin type A (alpha toxin-positive) (Aschfalk and Muller, 2002). Five large food poisoning outbreaks were described in which clinical, epidemiological or laboratory data indicated that *C. perfringens* from the boiled salmon was the causative organism (Hewitt et al., 1986). During 1983-1999, the causative agent was verified as *C. perfringens* in 14 outbreaks out of 1005 caused by seafood and fish products in Finland (Hielm et al., 2002). *Clostridium perfringens* was associated with two foodborne outbreaks related to fishery products in 2000 in Finland (Hatakka et al., 2001). The vehicles were traditional Finnish rye pastry filled with fish and pork (kalakukko), as well as hot-smoked and charred fish (Autio et al., 2004), and the main reasons for the outbreak were inappropriate temperature and prolonged storage time. No studies on *C. perfringens* in lampreys are available.

2.6.2 *Listeria monocytogenes*

Listeria monocytogenes is a species of the genus *Listeria* of the family *Listeriaceae* and a Gram-positive foodborne pathogen that causes listeriosis in humans. Listeriosis is more common in immunosuppressed and elderly people and therefore the mortality rate is high. A number of outbreaks caused by foodborne *L. monocytogenes* were confirmed with high mortality rates (Schlech et al., 1983; Linnan et al., 1988). *Listeria monocytogenes* in cold-smoked rainbow trout was associated with an outbreak of febrile gastroenteritis (Miettinen et al., 1999).

Listeria monocytogenes can also pass the foetoplacental barrier and cause complications during pregnancy. It has been recognized as an important foodborne pathogen since the early 1980s. As a significant causative agent of listeriosis *L. monocytogenes* has been studied intensively. Serotyping is the commonly used typing method and *L. monocytogenes* can be divided into 13 different serotypes (Seeliger and Hohne, 1979), of which the most common are 1/2a, 1/2b, 1/2c, 3a and 4b. The complex listerial pathogenesis in cells can be divided in to five stages: adhesis and invasion, lysis of the primary vacuole, intracellular growth, cell-to-cell spread and lysis of the double-membrane vacuole (Paoli et al., 2005). *Listeria monocytogenes* is commonly found in the environment and is also able to grow at refrigerated temperatures (Junttila et al., 1988; Walker et al., 1990; McClure et al., 1991; Bayles and Wilkinson, 2000).

The prevalence of *L. monocytogenes* in fresh, unprocessed, farmed rainbow trout in fish farms was an average of 14.6% (Miettinen and Wirtanen, 2005). Listeria are destroyed by cooking and inactivated by heating at 50 °C, and due to possible postprocessing contamination minimally processed foods are a significant concern. The association between *L. monocytogenes* strains in environmental samples from production plants and from fishery products sold in retail was verified (Johansson et al., 1999). Autio et al. (1999) studied the sources of contamination in a cold-smoked rainbow trout processing plant. Contamination of *L. monocytogenes* in fish processing plants can be prolonged or persistent for years (Hoffmann et al., 2003). The contamination mechanism and properties of persistent *L. monocytogenes* strains were analysed in food processing plants (Lundén, 2004). In a study (Thimothe et al., 2004) conducted in smoked-fish processing plants, *L. monocytogenes* was present in 4.8% of the environmental samples from food contact surfaces but in 12.8% of those from nonfood contact surfaces and 23.7% of drain samples. In samples from foodhandlers' contact surfaces (aprons, doorknobs and hands) 10.4% were positive, as were 1.3% of samples from the final product. The disparity between the subtypes found on raw fish and those found in the processing environment indicates that in processing plants there are several contamination routes.

The fishery products most reported to be involved with *L. monocytogenes* are cold-smoked, gravad and hot-smoked fish with prevalences of 6-50%, 4-27% and ND-9%, respectively (Lundén, 2004). The prevalence in retail market products was 22%. Beaufort

et al. (2004) found a prevalence of 10% in vacuum-packed cold-smoked salmon. Vacuum-packed smoked salmon stored at 4 °C was tested for the presence of *Listeria* spp., including *L. monocytogenes*, and growth in naturally contaminated salmon did not attain levels of 100 CFU/g within 4 weeks' storage (Lappi et al., 2004). In four smoked-fish processing plants *L. monocytogenes* was present in 16.7% out of 234 raw fish samples and 9.0% out of 233 samples of the final product. (Thimothe et al., 2004). *Listeria monocytogenes* has not been described in lamprey products.

2.6.3 *Staphylococcus aureus*

Staphylococcus aureus is a species of the genus *Staphylococcus* of the family *Staphylococceae.* These bacteria are found ubiquitous on mucous membranes and the skin of humans and animals. *Staphylococcus aureus* is a poor competitor and other organisms outgrow it when present (Casman et al., 1963). Therefore, food items that have been heated before being subjected to improper handling causing postprocessing contamination and poor refrigeration may allow the growth of *S. aureus*. Food poisoning caused by *S. aureus* is a result of the enterotoxins that certain strains are able to produce; ingestion of the organism itself is not necessary. Therefore, the detection of enterotoxin in the food item has discriminatory power. Several serological tests are available for detection of enterotoxin. PCR techniques can be used for screening for possible enterotoxin genes if the bacteria can be isolated from the sample.

Enterotoxigenic *S. aureus* strains were found in 30% or more in samples of healthy people, especially in their nasal cavities (Lina et al., 2003; Stewart, 2005). The proportion is even higher, up to 54%, among foodhandlers (Bergdoll, 1989; Hatakka et al., 2000). Foodhandlers, and not raw material such as fish, are the main reservoir of contamination.

Staphylococci are apparently adherent to processing equipment and are a persistent sanitation problem in processing plants. They were detected in a caviar plant not only before, but also after cleaning and disinfecting procedures (Bagge-Ravn et al., 2003). Wieneke et al. (1993) examined strains of *S. aureus* from 359 outbreaks and 79% of these strains produced enterotoxin; fish and seafood were the vehicle in 7% of cases.

Staphylococcus aureus was found in amounts of 5.0 log CFU/g in hot-smoked trout fillets (Kleickmann and Schellhaas, 1979). High amounts (up to 6.78 log CFU/g) of *S. aureus* were present in two samples of hot-smoked and charred Baltic herring stored for 48-96 hours at 20 °C (Korkeala and Pakkala, 1988). Ayulo et al. (1994) isolated *S. aureus* from 20% of 175 fish samples examined, including 60% of samples of shellfish meat. In hot-smoked vacuum-packed trout fillets *S. aureus* was found in samples stored at 4 ºC after 20 days and at 8 ºC after 13 days (von Zorn et al., 1993). Gonzalez-Rodriguez et al. (2001) found levels of *S. aureus* of 3.0 log CFU/g in prepacked fish portions and that lower than 4.0 log CFU/g in vacuum-packed cold-smoked salmon and trout (Gonzalez-Rodriguez et al., 2002). In 23 out of 105 cases of foodborne outbreaks between 1983 and 1999 in Finland caused by seafood and fish products, the causative agent was *S. aureus* (Hielm et al., 2002). *Staphylococcus aureus* populations increased rapidly in salmon strips during slow smoking and drying processes at 29-31 °C (Eklund et al., 2004). The occurrence of *S. aureus* in charcoal-grilled lamprey products has not been previously studied.

3 AIMS OF THE STUDY

The objectives of the present study were to investigate the hygienic quality and food safety of river lamprey and lamprey products with regard to residues, microbial spoilage and foodborne pathogens. The specific aims of this thesis were as follows:

1. to determine the residues of Hg, Cd and Pb in raw lampreys caught in several rivers in Finland (I),
2. to determine the PCBs and organochlorine pesticides in raw lampreys caught in several rivers in Finland (II),
3. to investigate the microbial levels in charcoal-grilled lampreys produced in three different plants and stored at 3 ºC and 22 °C (III),
4. to study microbial development and spoilage in vacuum-packed charcoal-grilled lamprey products (IV) and
5. to study the occurrence of *Clostridium botulinum* in raw lampreys and *Clostridium perfringens, Listeria monocytogenes* and *Staphylococcus aureus* in charcoal-grilled lampreys (III ,V).

4 MATERIALS AND METHODS

4.1 Sampling

4.1.1 Sampling for residues of mercury, cadmium, lead, PCBs and pesticides (I, II))

A total of 51 fresh lamprey samples were caught for Hg, Cd and Pb analyses during the period from September to October 1997 in nine different rivers and five delta areas downstreaming to the Bothnian Bay and the Bothnian Sea. Each sample consisted of six lampreys. Fifty fresh lamprey samples were caught for PCB and organochlorine residue analyses during the same period in 13 different rivers flowing in to the Bothnian Bay and the Bothnian Sea: 31 and 19 samples, respectively, each consisting of six lampreys. Before analysis the lampreys were homogenized and stored in a freezer (-18 °C).

4.1.2 Sampling for microbiological analyses (III, V)

For microbiological quality 10 production lots were collected from each of three manufacturers (A, B and C) during autumn 1998. Each lot consisted of 20 lampreys and was divided into two parts and placed in plastic bags permeable to the air under aseptic conditions. The time elapsed from collection of the lampreys to initiation of the storage study did not exceed 2 hours. Half of the samples were kept at 22 °C and the other half at 3 °C. These samples were studied 0, 2, 4 and 6 days or until the CFU levels stopped increasing and after 4, 8, 12, 16, 20 and 24 days, respectively. The sample studied consisted of a single whole lamprey. A total of 300 lampreys were studied.

To determine the level of microbial spoilage developing in vacuum-packed charcoal-grilled lamprey, a total of 450 lampreys were collected from five different production lots of each of three plants. These 15 samples were all divided into eight subsamples that were vacuum-packed using a VAC STAR 6500 ST packaging machine (Vac Star; Kerzens, Switzerland), in polyamide-polyethylene film (Suomen Union Verpackungs; Östersundom,

Finland) with an oxygen permeability of 25 ml O_2 per m^2 per 24h/atm [23 °C, 75% relative humidity (RH)] and a water vapour permeability of 2.5 g per m^2 per 24 h (23 °C, 85% RH). This resulted in 120 packages, 40 from each plant, which were studied in the sensory and microbiological analyses associated with the different storage time points at 8 °C.

For determining the presence of *C. botulinum* spores, a total of 67 raw lampreys were collected from 12 different rivers (Kalajoki, Iijoki, Pyhäjoki, Lapväärtinjoki, Ahlaistenjoki, Oulujoki, Perhonjoki, Kemijoki, Kiiminkijoki, Kokemäenjoki, Kuivajoki and Siikajoki) flowing into the Gulf of Bothnia in autumn 2003 and 2004. The lampreys were killed, frozen and stored at -18 °C until analysis. For the other pathogens sampling was done as for studies of microbiological quality.

4.2 Determination of mercury, cadmium, lead, PCB and pesticide residues (I, II)

4.2.1 Determination of mercury

The Hg content was determined with atomic absorption spectrophotometry (AAS) cold steam method (Lindsjö and Riekkola, 1976). The samples were digested with nitric acid (J.T. Baker; Deventen, Holland), sulphuric acid (J.T. Baker) and perchloric acid (Merck; Darmstadt, Germany) and the organic Hg was reduced to metallic Hg with stannium chloride dihydrate (J.T. Baker). The apparatus used for spectrometry was a Unicam 929 Atomic Absorption Spectroscopy Solar System (Cambridge, UK).

4.2.2 Determination of cadmium and lead

The Cd and Pb contents were determined with AAS (Anon. 1991a; Welz, 1983) after wet digestion (72 hours) in a thermal bath. The apparatus used was a Philips 1702 PU 9200 (Cambridge, UK) equipped with a graphite furnace. The samples were processed with nitric acid (Merck) and 30% hydrogen peroxide (J.T. Baker) Up to three replicate samples of each catch were examined.

4.2.3 Determination of PCBs and pesticide residues

The HCHs (alpha-HCH, beta-HCH, lindane), HCB, heptachloroepoxide, dieldrin, chlordanes (oxychlordane, trans-chlordane, cis-chlordane, TNCL), DDT compounds (p,p'-DDE, p,p'-DDD, p,p'-DDT, o,p'-DDT) and 15 PCB congeners were determined from all samples. The method used was an in-house modification of the methods described by the AOAC International (formerly Association of Official Analytical Chemists) (AOAC, 1995) and Specht (1987).

In all, 10 g of homogenized sample were mixed with sodium sulphate and sand and blended in a mortar until homogeneity was achieved. Extraction was performed twice with hexane-acetone (2+1) in a rotary shaker. After filtration the organic phase was evaporated and the lipid residue weighed. The pesticides were removed from the lipid with automated gel permeation chromatography. The elution solvent was dichloromethane-cyclohexane (1+1). Further cleanup and separation were achieved using a 3% deactivated silica gel column. The compounds were eluted with hexane (fraction 1, with PCBs and some organochlorine pesticides) and toluene (fraction 2, with organochlorine pesticides). The lipid content was determined gravimetrically according to a standard procedure after extraction of the sample with diethyl ether and light petroleum.

The PCBs and organochlorine pesticides were determined using a capillary gas chromatograph equipped with dual column and dual electrochemical detector systems. The gas chromatograph used was an Agilent 6890 Series (Agilent Technologies Inc., Palo Alto, CA, USA) and the capillary columns used were a DB-1701: 30 m, 0.25 mm i.d., 0.25 µm

film thickness and a DB-1: 30 m, 0.25 mm i.d., 0.25 µm film thickness. The following conditions were used: helium at a flow rate of 2.3 ml/min and oven temperature programmed to start at 80 °C, at which it was held for 2 min, followed by a linear increase to 190 °C at a rate of 30 °C/min at which the temperature was held for 13 min. The temperature was then linearly increased to 220 °C at a rate of 3 °C /min and held there for 2 min. Finally the temperature was increased to 260 °C at a rate of 10 °C/min and held there for 10 min. The injection volume was 2 µl and a splitless mode was applied. The organochlorine compounds were quantified against a five-point calibration curve; the final results were not corrected with recoveries.

4.3 Microbiological analyses (III-V)

4.3.1 Determination of APCs and LAB (III, IV)

A 10-g transversal slice sample of a lamprey was placed in a sterile stomacher bag (Seward Limited, London, United Kingdom) with 90 ml of a saline-peptone solution (0.85% NaCl, 0.1% peptone) (Maximal Recovery Diluent, Lab M Ltd., Bury, England) and homogenized with a stomacher (Lab-Blender 400, London, UK). The homogenized sample was serially diluted using 0.9-ml saline-peptone solution (Lab M Ltd.) dilution blanks. Each dilution was plated onto the appropriate media using the poured or spread plate technique, depending on the method used.

The APC was determined with the international standard method [International Organization for Standardization (ISO) 4833, 1991)] using plate count media (Oxoid, Hampshire, Basingstoke, UK). The presence of LAB was determined using the method of the Nordic Committee on Food Analysis (NCFA, 1991), modified by omitting sorbic acid and adjusting the pH to 5.7; the pH of the deMan, Rogosa, Sharpe medium (MRS, Oxoid) was 6.2. The presence of enterococci was determined with the NCFA method (NCFA,1992a), using Slanetz and Bartley agar (Oxoid).

4.3.2 Determination of *C. botulinum, C. perfringens, L. monocytogenes* and *S .aureus* (III,V)

For *C. botulinum* analysis twenty 1-g aliquots of each sample were transferred to tubes containing 10 ml of tryptose-peptone-glucose-yeast extract (TPGY) medium (Difco Laboratories, Detroit, MI, USA). The tubes were incubated anaerobically at 30 °C or 37 °C for 3 days, followed by subculture in fresh TPGY under the same conditions. Cells from 1 ml of each overnight culture were washed with 1 ml of TE buffer (0.01 M Tris-HCl, 0.001 M ethylendiaminetetraacetic acid (EDTA) for 1 h at 37 °C and suspended in 1 ml of distilled water. The suspensions were heated at 99 °C for 10 min to break up the cells and release the bacterial DNA, and were then centrifuged for 3 min at 13 000 x g. A 1-µl volume of each supernatant was used as a template in the PCR mixture. A multiplex PCR targeting the genes encoding botulinum neurotoxin types A, B, E and F was used (Lindström et al., 2001).

Clostridium botulinum was isolated from the PCR-positive overnight culture tubes by plating 0.1 ml of each culture on egg yolk agar (EYA) plates (Hauschild and Hilsheimer, 1977) and incubating the plates at 30 °C under anaerobic conditions for 2 days. The lipase-positive colonies were streaked onto fresh EYA plates followed by a 2-day incubation at 30 °C. Subculturing onto fresh EYA was continued until pure cultures were obtained. We confirmed that the cultures carried the type E toxin gene, using multiplex PCR (Lindström et al., 2001). One PCR-positive colony from each pure culture was permitted to sporulate by transferring it into 10 ml of anaerobic TPGY broth and incubating at 30 °C for 2 weeks. The spores were washed, with a final suspension in sterile distilled water. The spore suspensions were stored at 4 °C until PFGE analysis. The estimate for the *C. botulinum* count in the positive sample was determined using the most-probable-number (MPN) method (Thomas, 1942), with PCR-positive tubes being considered as positive.

For determination of *C. perfringens*, *L. monocytogenes* and *S. aureus* a 10-g transversal slice sample of a lamprey was placed in a sterile stomacher bag (Seward Ltd.) with 90 ml of saline-peptone solution (0.85% NaCl, 0.1% peptone) (Maximal Recovery Diluent, Lab M Ltd.) and homogenized with a stomacher (Lab-Blender 400). The homogenized sample

was serially diluted using 0.9-ml saline-peptone solution (Lab M Ltd.) dilution blanks. Each dilution was plated onto the appropriate media using the poured or spread plate technique, depending on the method used.

The presence of *C. perfringens* was determined using tryptose sulphite cycloserine (TSC) agar (Oxoid) (NCFA, 1997). *Clostridium perfringens* EELA 3/96 was used as a positive control. Isolation of *L. monocytogenes* was carried out according to the NCFA method (NCFA, 1990) using an Oxford Listeria-selective agar plate (Oxoid) and *L. monocytogenes* (EELA) as a positive control. The number of *S. aureus* was determined using Baird-Parker agar (Oxoid) (NCFA, 1992b) and confirmed using apiSTAPH (bioMérieux, Marcy d'Etoile, France). *Staphylococcus aureus* SLV-350 was used as a positive control.

4.3.3 Identification, selection and rRNA gene ribotyping of LAB (IV)

Identification of LAB

LAB were enumerated by plating of the samples on MRS Agar (Oxoid). The medium was inoculated using the spread plate technique and the plates were incubated under an anaerobic CO_2 atmosphere (Anaerogen, Oxoid) at 20 °C for 5 days. Duplicate plates were incubated under aerobic conditions. From each sample, randomly picked colonies were cultured, using MRS broth and MRS agar (Oxoid). A total of 27 colonies were selected from the plates, and subjected to species-level identification. The colonies were selected from the plates of the two highest dilutions showing growth. For DNA extraction, MRS broth was inoculated into tubes and grown at 25 °C for 1-2 days, depending on the growth rate. After incubation, cells harvested from 1.5 ml of the MRS broth were used for DNA extraction. If needed, the isolates were stored in MRS broth at -70 °C.

DNA isolation and ribotyping

DNA was isolated using the modified (Björkroth and Korkeala, 1996a) guanidium thiocyanate method of Pitcher et al. (1989). In this modification, the cell lysis solution contains mutanolysin (250 U/ml; Sigma, St. Louis, MO, USA) in addition to lysozyme (25

mg/ml, Sigma). Restriction endonuclease treatment of 8 µg of DNA was performed using (*HindIII*) restriction enzyme (New England Biolabs, Beverly, MA, USA) as specified by the manufacturer. *Hin*dIII was chosen because it provides species-specific patterns for various spoilage LAB (Björkroth and Korkeala, 1996b, 1997; Björkroth et al., 1998, 2000). Restriction endonuclease analysis (REA) was performed as described previously (Björkroth and Korkeala, 1996a). Genomic blots were made using a vacuum device (Vacugene; Pharmacia, Uppsala, Sweden) and the rRNA gene probe for ribotyping was labelled by reverse transcription [avian myeloblastosis virus-reverse transcriptase (AMV-RT), Promega, Madison, WI, USA] and Dig DNA Labeling Kit; Roche Molecular Biochemicals, Mannheim, Germany) as previously described by Blumberg et al. (1991). The membranes were hybridized at 58 °C overnight and detection of the digoxigenin label was performed as recommended by Roche Molecular Biochemicals.

Use of LAB database for identification

The membranes were scanned with a Hewlett-Packard ScanJet 4c/T tabletop scanner (Boise, ID, USA). Numerical analysis of the ribopatterns was performed using the Bionumerics 3.0 software package (Applied Maths, Sint-Martens-Latem, Belgium). Based on internal controls, 1.5% position tolerance and 0.5% optimization were allowed for the bands/patterns. The similarity between all pairs was expressed by Dice coefficient correlation, and the unweighted pair-group method with arithmetic averages (UPGMA) was used for the construction of the dendrogram. The ribopatterns were compared with the corresponding patterns in the LAB database at the Department of Food and Environmental Hygiene, University of Helsinki, Finland. It comprises patterns of all relevant spoilage LAB in the genera *Carnobacterium*, *Lactobacillus*, *Leuconostoc*, *Enterococcus* and *Weissella* (Björkroth and Korkeala, 1996b, 1997; Björkroth et al., 1998, 2000). Identification of the isolates was performed, based on the locations of the type strains in the clusters.

4.3.4 Sequencing of 16S rRNA gene (IV)

In case ribotyping database analysis did not result in clear clusters, representative strains from these clusters were subjected to 16S rRNA gene sequence analysis. The nearly complete 16S rRNA gene was amplified by PCR with a universal primer pair F8-27 (5'-AGAGTTTGATCCTGGCTGAG-3') and R1541-1522 (5'-AAGGAGGTGATCCAGCCGCA-3'). Bidirectional sequencing of the purified (QIAquick PCR Purification Kit; Qiagen, Valencia, CA, USA) PCR product was performed with Sanger's dideoxynucleotide chain termination method, using two long primers [F19-38 (5'-CTGGCTCAGGAYGAACGCTG-3') and R1541-1522] and two shorter reaction primers [F926 (5'-AACTCAAAGGAATTGACGG-3') and R519 (5'-GTATTACCGCGGCTGCTG-3')]. The samples were run in a Global IR2 sequencing device with e-Seq 1.1 software (LiCor, Lincoln, NE, USA) according to the manufacturer's instructions. The similarity between these sequences and the sequences deposited in GenBank was compared with BLASTN 2.2.6 software (Altschul et al., 1997).

4.3.5 Pulsed-field gel electrophoresis of *C. botulinum* isolates (V)

For the PFGE analysis, 100 µl of each spore suspension were streaked onto fresh EYA and incubated anaerobically at 30 °C for 2 days. One colony was further transferred to 10 ml of anaerobic TPGY medium and incubated anaerobically at 30 °C for 24 hours, followed by anaerobic overnight culture in fresh TPGY at 30 ºC for 16-18 hours. The PFGE analysis was performed from 8 ml of the overnight cultures as previously described (Hielm et al., 1998c), with the following modifications. A 20-min formaldehyde fixation of cells prior to lysis was employed to avoid DNA degradation. The 20 x EDTA-N-lauroylsarcosine-proteinase K (ESP) buffer used for completion of cell lysis contained 0.5 M EDTA (pH 8.0), 10% sodium lauroyl sarcosine and 20 mg/ml pronase (Pronase; Roche Diagnostics GmbH, Mannheim, Germany). Pefabloc SC (Roche) at a concentration of 25 ng/ml was used to inactivate the pronase activity. Restriction enzymes (*Sma*I) and (*Xho*I) (New England Biolabs) were used to cleave the DNA. In the electrophoresis, the Low-Range PFG Marker (New England Biolabs) was used and the pulse time ramped from 1 to 26 sec for 22 hours at 200 V.

4.4 Sensory evaluation (IV)

To determine when the products studied were spoiled, the samples were evaluated once per week for taste by a trained panel that was familiar with off-flavour problems typical for lampreys. The samples were evaluated for aroma and taste using the method described by Amerine et al. (1965). All samples were coded by number and were presented to the panel in randomized order. The scale for taste was 0-10 and arithmetic means were calculated from individual scores.

4.5 Determination of NaCl concentration and a_w (IV)

The NaCl concentration, expressed as %NaCl, was determined by potentiometric titration. In addition, a separate experiment was performed to determine the water activity (a_w) (NCFA, 1984) as a general indication of product composition.

4.6 Statistical analysis (I, IV)

Student's t-test and χ^2-test were applied to calculate differences between plants in the APC, LAB and sensory scores, and the Student's t-test to analyse the residue data.

5 RESULTS

5.1 Mercury, cadmium, lead, PCB and pesticide residues in raw lamprey (I, II)

The mean content of Hg was 96 µg kg^{-1} in catches from the Bothnian Bay, which was higher than the of 49 µg kg^{-1} in lampreys from the Bothnian Sea ($p < 0.001$). The mean concentration of Cd in samples from the Bothnian Bay was 50 µg kg^{-1}, whereas from the Bothnian Sea it was significantly lower at 34 µg kg^{-1} ($p < 0.01$). The highest concentration found for Hg was 136 µg kg^{-1} (Iijoki) and for Cd 97 µg kg^{-1} (Siikajoki). The Pb content was under the detection limit in all samples except one caught in the Kokemäenjoki (64 µg kg^{-1}).

The pesticide residues in the rivers are presented in Table 2. The mean concentrations of the indicative PCB congeners were: PCB 28 1.4, PCB 52 1.0, PCB 101 6.4, PCB 118 10.0, PCB 138 19.5, PCB 153 30.0 and PCB 180 12.0 µg kg^{-1}. The mean ΣPCB value in samples from rivers flowing downstream to the Bothnian Bay was 110 µg kg^{-1} which was significantly lower than the 130 µg kg^{-1} in samples from the Bothnian Sea area ($p < 0.01$). The maximum values for ΣPCB in the northern and southern areas were 160 µg kg^{-1} and 180 µg kg^{-1}, respectively. The mean concentrations of individual congeners of 128, 149, 151, 170 and 180 were statistically higher in lampreys caught from the southern area than in the northern area ($p < 0.01$). The predominant congeners were 138 and 153. The level of dioxinlike PCB congeners 114 and 118 did not differ in the two catchment areas. Congener 15 was not detected in any sample. The mean fat concentrations were 15.2% in river lampreys from the Bothnian Sea and 16.1% from the Bothnian Bay rivers.

The mean concentrations of alpha-HCH, beta-HCH, cis-chlordane, DDT compounds (p,p'-DDE, p,p'- DDD, p,p'- DDT, o,p'- DDT), dieldrin, HCB, heptachloroepoxide, lindane, oxychlordane, trans-chlordane and TNCL were in samples from the Bothnian Bay and Bothnian Sea 1.2 and 1.6, 0.63 and 1.1, 2.7 and 3.1, 46 and 68, 3.7 and 5.3, 3.1 and 4.4, 0.74 and 0.86, 1.2 and 1.5, 1.1 and 1.3, 0.49 and 0.41, 9.5 and 9.8 µg kg^{-1} fresh weight, respectively.

The concentrations of DDT were significantly higher in lampreys caught from rivers in the southern area than in those from the northern area ($p < 0.01$); the maximum values were 87 and 66 $\mu g/kg^{-1}$ respectively. The mean concentrations of beta-HCH, HCB, dieldrin and oxychlordane were significantly higher in samples from rivers flowing downstream to the Bothnian Sea in comparison to the Bothnian Bay ($p < 0.01$). The mean residues of lindane were also lower in the northern area ($p < 0.05$). There was no difference between the areas in the concentrations of alpha-HCH, heptachloroepoxide, trans-chlordane, cis-chlordane and TNCL.

Table 2. Mean residue levels of Σ PCB and some pesticides in lampreys from different rivers flowing downstream to the Gulf of Bothnia (µg kg^{-1} fresh weight).

		Pesticide											
River	n	Σ PCB	Alpha-HCH	Beta-HCH	Cis-chlordane	Σ DDT	Dieldrin	HCB	Heptachloro-epoxide	Lindane	Oxy-chlordane	Trans-chlordane	Trans-nonachlor
Bothnian Bay													
Kemi	1	130	0.84	0.75	4.1	58	4.2	2.2	0.56	0.87	0.82	0.61	11
Ii	5	110	1.1	0.37	2.7	46	4.4	2.5	0.74	1.2	1.2	0.41	13
Kiiminki	6	110	1.6	0.88	2.8	47	4.1	3.4	0.87	1.3	1.0	0.53	8.1
Siika	10	110	1.1	0.49	2.5	45	3.3	2.7	0.72	1.1	1.1	0.45	9.1
Pyhä	5	110	1.1	0.80	2.4	47	3.9	3.5	0.75	1.2	1.0	0.60	7.7
Kala	1	120	2.2	1.7	3.0	45	2.0	5.6	0.69	1.7	1.0	0.43	11
Lesti	1	110	1.4	0.36	2.8	48	2.9	5.2	0.59	1.3	1.0	0.49	10
Perho	2	100	1.3	0.41	2.6	45	3.2	2.9	0.66	1.1	1.2	0.36	11
Bothnian Sea													
Kyrö	1	120	0.45	0.19	3.0	72	2.4	4.2	1.0	1.2	1.1	0.087	13
Tiukka	3	120	1.2	0.99	2.1	56	5.0	3.6	0.91	0.89	0.94	0.28	6.5
Lapväärtti	5	120	1.7	1.1	3.0	61	4.9	3.8	0.80	1.4	1.2	0.46	6.8
Ahlainen	6	130	2.0	1.5	3.3	78	5.9	4.1	0.74	1.9	1.6	0.44	13
Kokemäki	4	140	1.4	1.1	3.6	71	6.0	6.2	1.1	1.5	1.4	0.46	11

5.2 Spoilage of charcoal-grilled lamprey (III, IV)

5.2.1 Product stored at 3 and 22 °C (III)

The mean APC of charcoal-grilled river lamprey on the day of production in samples from plant A was 2.29 log CFU/g and from plant B 1.88 log CFU/g. In samples from plant C the growth in every production lot was under the detection limit of 2.0 log CFU/g. After storage for 2 first days at 22 °C the mean APCs in the product did not exceed 2.82 , 2.29 and 2.82 log CFU/g in plants A, B and C, respectively. The mean APCs in samples from plant A stored at 22 °C were on day 4 and 6 significantly higher than in those from plants B and C ($p < 0.05$). There were no statistically significant differences in bacterial mean log counts between plants B and C. The percentage of samples with APCs over 7.0 log CFU/g after 4 days´ storage were 60%, 10% and 30% in samples from plants A, B and C, respectively. After 6 days' storage they were 60%, 40% and 40%, respectively. There was a significant difference between all producers after 4 days' storage and after 6 days between A and B and between A and C ($p < 0.05$). The numbers of samples in which the APC exceeded the detection limit for counts in samples from plant A stored at 22 °C were statistically higher in comparison to plants B and C ($p < 0.05$).

In lampreys stored at 3 °C the maximum mean APC were 2.82 log CFU/g and 2.62 log CFU/g, in samples from plants A and B, respectively. In samples from plant C, the APC was under the detection limit in all samples. One production lot from plant A exceeded the value 8.0 log CFU/g after 4 days' storage. There were no statistical differences in APC counts between the different plants. The percentage of samples exceeding the detection limit (2.0 log CFU/g) in plants A, B and C were 15%, 8% and 0%, respectively. The APCs in samples from plant C were statistically lower in comparison to those from A and B ($p < 0.05$). No enterococci were found during storage at 20 °C or at 3 °C.

5.2.2 APC, LAB strains and sensory evaluation in vacuum-packed lamprey product (IV)

The APC counts during storage at 8 °C are presented in Fig. 1. After one week's storage at 8 °C, the APC was under 3.0 log CFU/g in all samples from every plant. During 2-8 weeks' storage the mean APC increased in samples from plants A and C to 6.01 and 4.87 log CFU/g, respectively, but were under 3.0 log CFU/g in samples from plant B. Five samples from plant A and one from plant C exceeded the APC value of 7.0 log CFU/g after 6 weeks. In every sample from plant B, the APC was under 7.0 log CFU/g. The APCs in lamprey samples stored 4 and 8 weeks at plant A were higher than in plant B ($p < 0.05$ and $p < 0.01$, respectively).

The LAB counts are presented in Fig. 2. After 2 weeks' storage the LAB counts were under 3.0 log CFU/g in all samples from every plant. By weeks 6-8 the mean LAB counts varied between 4.07 and 4.86 CFU/g in samples from plant A and between < 2.7 and 5.48 log CFU/g in those from plant C. In samples from plant B no growth of LAB was detected. The proportion of LAB compared with APCs in samples from plant A was 53% after storage for 3 weeks, but this diminished after 6 and 8 weeks to 25% and 7%, respectively.

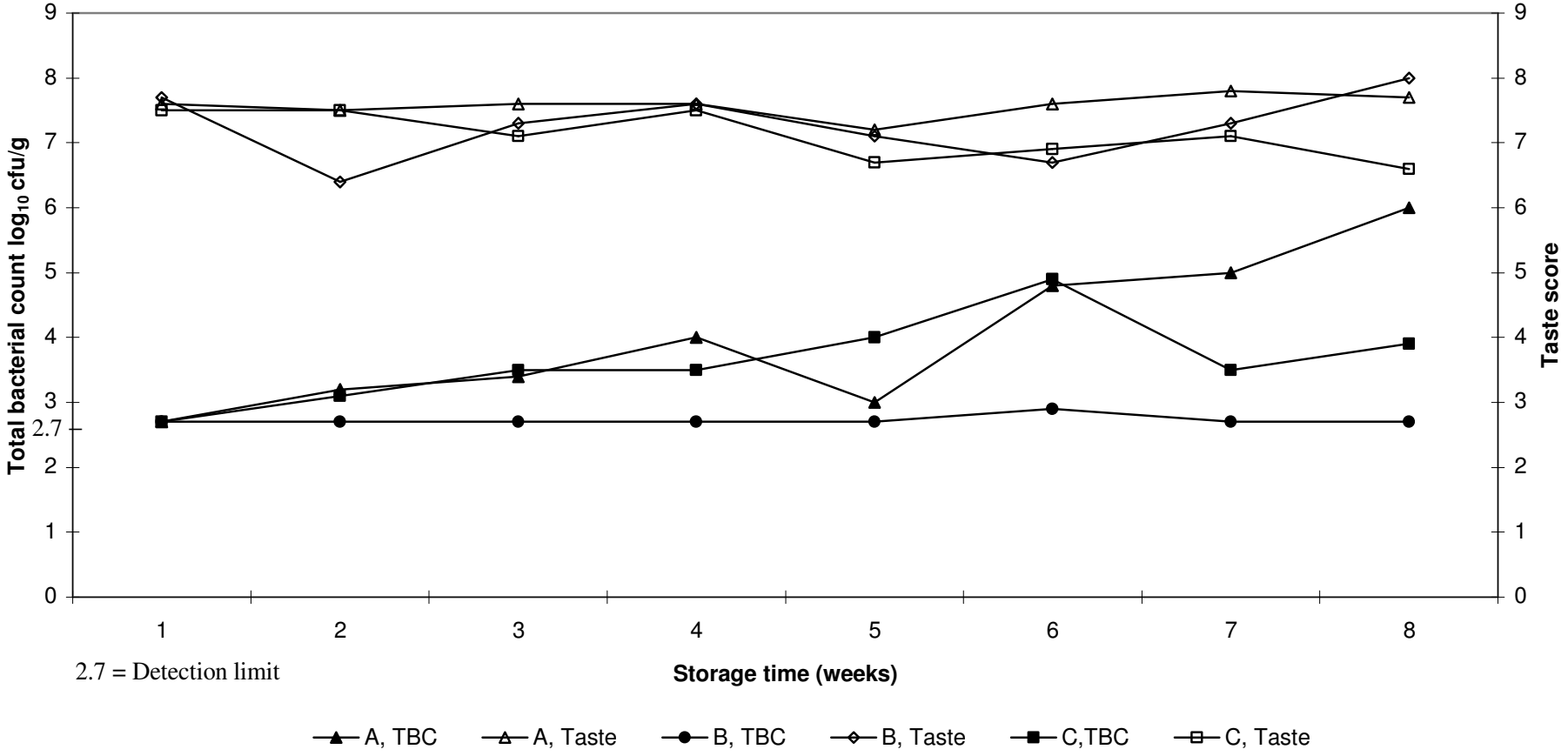

Figure 1. Total bacterial counts and taste scores in samples of vacuum-packaged charcoal-broiled river lamprey from three different plants (A, B and C) during eight weeks' storage.

Based on the isolates obtained in the samples from two plants, A and C, five different ribotypes were found. Ribotypes I (one isolate) and IV-V (2 and 12 isolates, respectively) were isolated from samples originating from plant A. Twelve isolates (ribotype V) from plant A possessed patterns identical with *Lactobacillus curvatus* subsp. *curvatus* and two (IV) with *Leuconostoc mesenteroides* subsp. *dextranicum* or *L. mesenteroides* subsp. *mesenteroides*. One isolate (I) showed a ribotype similar to that of *Weissella halotolerans*. Twelve isolates possessing two different patterns were not identified to the species level in the restriction fragment length polymorphism (RFLP) Lab database. Of these isolates two were selected to represent this group and were sequenced for their 16S rRNA genes. Representative strains of the nine isolates (ribotype III) from plant C showed 99.9% similarity to *Staphylococcus warneri*, type strain American Type Culture Collection (ATCC) 27836. Two from plant A (II) and one from plant C (II) showed 99.9% similarity to *Staphylococcus pasteuri* type strain ATCC 51129.

After storage for 8 weeks the lampreys still received good sensory scores from the panel (Figs. 1 and 2). The scores decreased slightly only in samples from plant C. There was no association observed between taste scores and APC or LAB counts. The NaCl concentration of the product was 2.5% (1.5-3.4%) and the a_w values tested varied between 0.93 and 0.98.

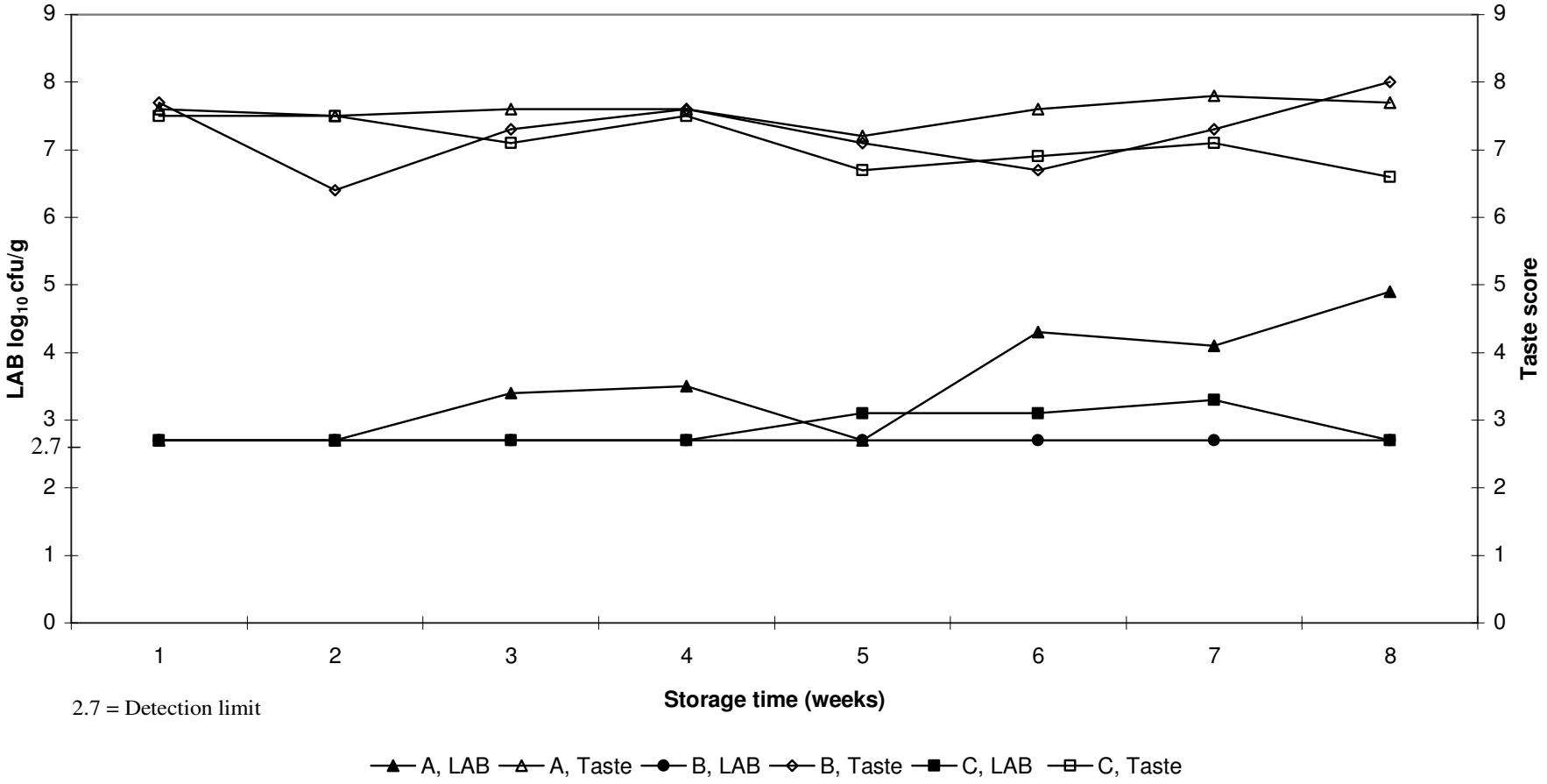

Figure 2. LAB counts and taste scores of vacuum-packaged charcoal-broiled river lamprey from three different plants (A, B and C) during eight weeks' storage.

5.3 Bacterial pathogens in raw lampreys and charcoal-grilled lampreys (III, V)

One raw lamprey sample out of 67 (1.5%) contained *C. botulinum* type E. With the positive sample, two 1-g subsamples out of 20 were positive, suggesting the *C. botulinum* count to be 100 spores per kg. The PFGE patterns of the lamprey isolates were different from the type E strains previously isolated from Finnish fish and sediment samples.

No *C. perfringens* or *L. monocytogenes* were detected in any of the charcoal-grilled lamprey samples studied. *Staphylococcus aureus* was isolated in one sample (0.3%) from a product from plant A stored for 4 days at 3 °C (3.57 log CFU/g) and in one sample (0.3%) from plant B stored for 2 days at 22 °C (4.95 log CFU/g).

6 DISCUSSION

6.1 Residues (I, II)

6.1.1 Mercury, cadmium and lead (I)

The study showed that the concentrations of Hg and Cd found in river lampreys were higher in the Bothnian Bay than in the Bothnian Sea, possibly be due to the metal industry and construction of large artificial lakes for hydroelectric power stations in the drainage basins of the northern rivers. The average concentrations of Hg and Cd are 2-4 times higher in the surface sediments of the Bothnian Bay than in the Bothnian Sea (Leivuori and Niemistö, 1993). The predominant emission of heavy metals from the Swedish smelter at Rönnskär in the Bothnian Bay decreased significantly from the levels present in the early 1970s (Anonymous, 1991b). In 25 years the average levels of Hg in lamprey have decreased in catches from the Bothnian Bay by more than four times: to 96 µg kg^{-1} compared with 400 µg kg^{-1} in a previous study (Kukko and Turunen, 1973). This is probably due to diminished industrial emissions to the air and from the reservoirs in the artificial lakes. The Hg levels in all samples were below 500 µg kg^{-1} (average 73 µg kg^{-1}), the highest acceptable level for fish and fishery products in the EU (EC, 2005). In a recent study (Hansen and Gilman, 2005) the need was shown for better understanding of the interactions and benefits associated with marine foods, which may reduce the health risks associated with low-level Hg exposure. Consuming fish as advised may increase the exposure to Hg, showing that there is still need for international action to reduce Hg emissions.

Cd levels (34-50 µg kg^{-1}) did not exceed the EU regulation limit (50 µg kg^{-1}) (EC, 2005) but were very close, showing the need for reducing Cd emission to the environment. It appeared that Pb is not a hazard in lamprey. The Pb concentration found in only one sample (64 µg kg^{-1}) was similar to the detection limit of 40 µg kg^{-1} and below that found earlier in cod (86 µg kg^{-1}) in the southern Baltic Sea (Falandyz, 1986b) and clearly below

the EU regulation limit (200 µg kg^{-1}). Since the 1980s, the Pb concentrations in herring have generally decreased (HELCOM, 2007).

Even though the concentrations of some heavy metals have decreased in many parts of the Baltic Sea, high concentrations can still be found in certain marine organisms, notably in Baltic herring, on which lampreys mainly feed.

6.1.2 PCBs and pesticide residues (II)

The mean residues ΣPCB, ΣDDT, beta-HCH, alpha-HCH, lindane, HCB, dieldrin, and oxychlordane were higher in catches from rivers flowing into the Bothnian Sea than rivers flowing into the Bothnian Bay. The mean PCB values (110 µg kg^{-1} from the Bothnian Bay and 130 µg kg^{-1} from the Bothnian Sea) are lower than the 170 µg kg^{-1} in a study of Falandysz et al. (2002) in lamprey from the Gulf of Gdansk. The concentrations of cis-chlordane, trans-chlordane, TNCL, oxychlordane and dieldrin were similar to the level observed in this study in lampreys from the southern Baltic (Falandyz et al., 2001). The level of heptachloroepoxide was higher in samples from the Gulf of Bothnia than from the southern Baltic. In line with this study are the concentrations tested for beta-HCH, alpha-HCH, lindane, HCB, TNCL and ΣDDT in Baltic herring from the Bothnian Sea and Bothnian Bay (Nakari et al., 2004).

Nakari et al. (2002, 2004) monitored the residues of PCBs, DDT, and some pesticides in Baltic herring and northern pike from the Bothnian Bay and Bothnian Sea during 1997-1999 and 2000-2002. They showed that the concentrations of the analytes were higher in Baltic herring than in pike. The compounds measured are lipid-soluble and their concentrations were higher in fish species with higher fat content. In general the percentage of fat in pike, Baltic herring, and lamprey varies 0.36-0.43%, 2.3-3.50% and 15.2-16.1%, respectively. The high fat concentration together with high position in the food chain probably explains, at least partially, the higher amounts of various organohalogen residues in river lamprey than in Baltic herring.

The national authorities (Anonymous 2003) have set action limits for PCBs and pesticide residues in fish. In our study the maximum residue level in lampreys was 180 (action limit 2000) µg kg^{-1} for ΣPCB, 3.0 (action limit 200) µg kg^{-1} for lindane, 7.3 (action limit 200) µg kg^{-1} for HCB, 1.7 (action limit 100) µg kg^{-1} for heptachloroepoxide and 87 (action limit 500) µg kg^{-1} for ΣDDT. All residues studied were accordingly well below the action limits. The Helsinki Convention (1992) revised recommendations for 2004 (HELCOM, 2007), however, especially name PCBs as pollutants for which special bans and restrictions on transport, trade, handling, use and disposal are imposed. The concentration of PCBs in herring muscle from all herring sites in the Baltic tended to decrease significantly during the time period 1978/80-2002 (HELCOM, 2007).

The PCB concentrations were higher in Baltic herring from the Gulf of Bothnia than those in the Gulf of Finland, possibly due to greater gaps in weight between small and large herring or different feeding characteristic (Kiviranta et al., 2003). In the Gulf of Bothnia there is a higher pollution burden in the Bothnian Sea than the Bothnian Bay, and the Gävlebukten region in the Bothnian Sea is a point source for PCBs, HCBs and DDTs. The Gulf of Gdansk has a similar pollution level of organochlorine compounds compared with the background station or open sea situation in the Gulf of Bothnia, except for the elevated levels of DDTs and PCBs (Strandberg et al., 1998a). Since their complete ban in the 1980s, several POPs, notably certain organochlorine pesticides such as DDT and technical grade HCH, have considerably decreased in the waters of the Baltic Sea (HELCOM, 2007).

The differences seen in the levels of PCBs and organochlorine compounds in lamprey taken from the Bothnian Bay and Bothnian Sea may have resulted entirely for geographical reasons. Industrial sites are more commonly found in the southern regions, while the northern catchment area is wilderness with abundant forested land and less agriculture. The drainage basin for the northern rivers in Finland consists of approximately 329 000 ha of land under cultivation and the southern drainage basin of 679 000 ha (Ministry of Agriculture and Forestry, 2003). The total sale of fungicides in Finland has increased during 1996-2002 from 231.6 to 512.6 tonnes and that of herbicides from 1585.7 to 2929.8 tonnes. The sale of insecticides varied in the same period from 107.5 to 190.4 tonnes (Ministry of Agriculture and Forestry, 2003). Since the amount of land under cultivation is doubled in the southern area, it may be assumed that the use of pesticides is also higher.

The amount of forested land in the northern and southern drainage basins is similar: being 767 600 ha and 856 200 ha, respectively. The yearly use of pesticides in forested land has diminished during 1996-2002 from 45.3 tonnes to 8.5 tonnes (Ministry of Agriculture and Forestry, 2003). Thus the pesticide contamination probably originated from agriculture.

Even though the concentrations of organic compounds were higher in river lamprey than in pike and Baltic herring, lamprey appear to be a safe foodstuff with respect to the residues measured in this study. The present study implies that there are differences in the concentrations of some residues between different basins of the Gulf of Bothnia and gives information on safety evaluation of foodstuffs of marine origin.

6.2 Spoilage in charcoal-grilled lamprey (III, IV)

The microbiological quality of charcoal-grilled river lamprey varied, depending on the plant where they were processed. The mean APCs for samples stored at 22 °C in plant A were significantly higher than those of plants B and C ($p < 0.05$), possibly due to differences in the grilling procedure. The APCs were under the detection limit in samples from plant C stored at 3 °C, regardless of the time of storage, but there were no statistical differences between the APCs of other plants.

It is crucial to determine microbial development in grilled lamprey during the first 2 days of storage even at 22 °C, because the product is brought to the market without chilling. The APCs of charred river lamprey did not exceed 6.0 log CFU/g during the first 2 days, even when stored at 22 °C. After 4 days storage the APCs exceeded 7.0 log CFU/g in 60% of the samples, suggesting that the traditional practice of nonchilling results in deterioration of the product after 2 days.

The results indicate that microbial growth in vacuum-packed lamprey products is relatively slow. After storage for 3 weeks, the mean APC was 3.48 log CFU/g or less in the products of two plants and under the detection limit in the product of the third plant. After 8 weeks, the highest mean APC attained was 6.01 log CFU/g. Magnússon and Traustadóttir (1982) reported an APC in vacuum-packed smoked herring fillets of 6.0 log CFU/g in less than 3

weeks and > 7.5 log CFU/g after 6 weeks. In vacuum-packed hot-smoked trout filets stored at 8 °C the APC attained the value 6.0 log CFU/g after 7 days and sensory changes were present in 37% of samples (von Zorn et al., 1993). Thus, the lamprey product appears to remain edible for at least 5 weeks. The sensory scores remained almost at baseline levels after storage for 8 weeks, probably due to slow development of the microbial population. The product attained an APC of 7.0 log CFU/g after 6 weeks' or longer storage. The sensory panel stated that vacuum-packed smoked herring fillets (Magnússon and Traustadóttir, 1982) were still of high quality after storage for 12 weeks at 10 °C, although a rapid increase in microbes occurred during the first several weeks. The authors speculated that the high numbers of LAB found could have played a role in the long shelf life of the product. With regard to charcoal-grilled river lamprey, this does not appear likely. The different handling of lampreys may also affect the initial microbial population of the product. We also found that storing lampreys at 3 °C increased the shelf life of the product by up to 24 days, except one lot from plant A which apparently was not processed properly.

The results differ from findings obtained in studies investigating other fish products. Magnússon and Traustadóttir (1982) stated that LAB attained in vacuum-packed smoked herring at the expense of other genera, attaining a level of 76% after storage for 9 weeks. In hot-smoked tuna the LAB counts were 2.9 log CFU/g immediately after smoking and 9.0 log CFU/g after 10 weeks' storage in vacuum packs at 5 °C (Paleari et al., 1989). Lyhs et al. (1998) found that LAB predominated in the spoilage population of all samples from vacuum-packed cold-smoked rainbow trout fillets cured with NaCl. Spoilage is a result of the production of off-odours and -flavours often caused by bacterial activity. Since only part of the total bacterial population participates in the spoilage process, causing spoilage characteristics (Gram et al., 2002), the total number of microbes is not necessarily correlated with the presence of spoilage.

In charcoal-grilled lampreys the LAB did not appear to be an important group of spoilage bacteria. Among the LAB found in samples the predominant species in vacuum-packed charred lamprey was *L. curvatus* subsp. *curvatus*. Only two out of 27 isolates were identified as *Leuconostoc* spp. Jeppesen and Huss (1993) reported that all isolated LAB from samples of vacuum-packaged minced herring were *Leuconostoc* spp. Lyhs et al.

(1999) found *Leuconostoc mesenteroides* subsp. *mesenteroides, Leuconostoc citreum, Lactobacillus sakei* and *Lactobacillus curvatus* to be the main species associated with spoilage of vacuum-packaged cold-smoked rainbow trout. *Carnobacterium piscicola* were dominant LAB in cold-smoked salmon (Leroi et al., 1998) and in cold-smoked vacuum-packaged salmon (Paludan-Müller et al., 1998). Ribotyping revealed that only the products from plant A possessed LAB species; three different ribotypes were detected, most often *Lactobacillus curvatus* subsp. *curvatus*. Surprisingly, 10 strains in samples from plant C and two from plant A were identified as S*taphylococcus* spp. This shows that the MRS medium is not entirely selective for LAB. One ribotype (II) was common to both plants A and C.

In lampreys, the mean APC values being higher than the mean LAB values indicates that microbial groups other than LAB are also present. This may be due to the different processes involved, i.e. smoking versus charring, in the latter of which the relatively high temperatures (in average 153 °C) burn the surface of the product, resulting in a dry crispy coating. The values of a_w between 0.93 and 0.98 do not explain the slow microbial development, and the bacteria recovered in the product such as *Lactobacillus*, *Leuconostoc*, *Weissella* and *Staphylococcus* display growth potential at that range. Levels of a_w as low as 0.91 allow growth of microorganisms similar to those isolated here. Since the lampreys stop feeding at the beginning of the spawning season and their energy is deposited as fat, they may also constitute poor nutritional value for microbes, especially for LAB, which usually rely on fermentative carbohydrate metabolism.

6.3 Bacterial pathogens in raw lampreys and charcoal-grilled lampreys (III, V)

One raw lamprey sample (1.5%) contained *C. botulinum* type E. The contamination level for *C. botulinum* in nonfarmed raw fish was 23% in the Finnish catching areas (Hyytiä et al., 1998). The highest prevalence of *C. botulinum* type E was in Baltic herring, whitefish and burbot samples: 40%, 18% and 18%, respectively.

Nonproteolytic *C. botulinum* may grow and produce toxins at refrigeration temperatures (Graham et al., 1997). Fish products have been cited as causing botulism since the 1960s worldwide (Anonymous, 1964, 1968; Korkeala et al., 1998; Dawar et al., 2002; Boyer et al., 2001; Dressler, 2005). Since lampreys feed on herring and other fish species, but also spend 4-6 years in the bottom sediments of rivers during the larval stages, it is evident that they become contaminated with *C. botulinum* type E, probably by feeding on the fish. The positive lamprey sample contained two genetically distinct *C. botulinum* type E isolates. This finding confirms previous findings on the wide genetic diversity of *C. botulinum* type E (Hielm et al., 1998b; Hyytiä et al., 1999).

The mean minimum and maximum temperatures inside lampreys during grilling in one plant were 90 °C and 114 °C (Merivirta, 2006) and the time range between 16 and 23 min. This may be sufficient to reduce the number of spores of nonprotelytic *C. botulinum* spores to a safe level. The decimal reduction time for the heat-resistant spore fraction of type E was 7.1 min at 90 °C in whitefish media (Lindström et al., 2003). However, lampreys are processed in small-scale plants and the product core temperatures during the heating processes are not monitored. There is also variation in the processing practices between different producers, which may result in differences in temperatures affecting the inactivation of the *C. botulinum* spores. Hyytiä et al. (1998) showed that air-packed smoked lampreys (4%) may contain *C. botulinum* type E, indicating that the heat process employed in smoked lamprey production is not sufficient to destroy *C. botulinum* spores. The alternative explanation may be that postprocessing contamination could occur in small plants without separated production lines.

The a_w values tested in the lamprey product varied between 0.93 and 0.98. The inhibitory a_w for the growth and toxin production of *C. botulinum* type E is 0.97, corresponding to a brine concentration of 5% (w/v). Since the average salt concentration in the final lamprey products is about 2.5% (1.5-3.4%) (w/w), it is possible that *C. botulinum* type E may grow and produce toxin in these products under otherwise optimal conditions. Therefore, before vacuum-packing is employed in the lamprey industry, the safety of the packaged product should be corroborated by a challenge study. The presence of *C. botulinum* type E in the raw river lamprey sample suggests a potential safety risk related to the processed product. To control the potential risk of botulism related to vacuum-packaged charred lampreys, a

storage temperature of 3 °C or below combined with restricted shelf lives should be recommended for these products.

No growth of *C. perfringens* or *L. monocytogenes* was detected. This may have been caused by the heavy heat treatment (from 16 to 23 min at the oven temperature 100-293 °C) used on the product. River lampreys stop feeding at the beginning of their spawning migration. Therefore the intestinal content is minor and the microbial population low in lampreys caught during migration. This may partially explain the relatively low microbial development of the product even when stored at 22 °C.

Staphylococcus aureus was the only pathogenic bacteria isolated in the charcoal-grilled product. It was found in two samples (0.6% of the total), which was probably caused by contamination from workers' hands or utensils after processing. Hatakka et al. (2000) observed a prevalence of *S. aureus* in nasal samples (29%) and hand samples (9%) of flight-catering employees. Smoked and charred Baltic herring were reported to be a common cause of staphylococcal food poisoning outbreaks (Hirn and Aho, 1986). Since the lamprey product is not systematically chilled and stored at < 3-8 °C, but instead stored at room temperature, there is the possibility that *S. aureus* may grow during transport and retail and present a risk of food poisoning.

7 CONCLUSIONS

1. Hg and Cd were found in small amounts in lampreys. Lampreys caught in the Bothnian Bay area, the northern basin of the Gulf of Bothnia, had significantly higher levels of Hg and Cd than those caught in the Bothnian Sea. Over the last 30 years the Hg content of lampreys has decreased and the concentration was clearly under the highest acceptable level for fish and fishery products in the EU. Cd residues were at acceptable levels and Pb residues were of no significance in lampreys.

2. The mean residues for ΣPCB, ΣDDT, beta-HCH, alpha-HCH, lindane, HCB, dieldrin and oxychlordane were higher in catches from rivers flowing to the Bothnian Sea than rivers flowing to the Bothnian Bay. The differences seen in the levels of PCBs and organochlorine compounds in lamprey taken from the Bothnian Bay and Bothnian Sea areas may have resulted entirely from the varying physical characteristic of different drainage basins. The northern catchment area is wilderness with abundant forested land and less agriculture while industrial sites and agriculture are more common in the southern regions. All residues were well below the action limits set by the national authorities for PCB, lindane, HCB, heptachloroepoxide and DDT residues in fish.

3. The microbiological quality of charcoal-grilled river lamprey varied, depending on the plant. Lamprey processing methods vary in different plants. The traditional practice of nonchilling and storage at 22 °C results in deterioration of the product after 2 days, while chilling and storage of the product at 3 °C increases the shelf life of the product by up to 24 days.

4. Microbial development in vacuum-packed charred river lampreys stored at 8 °C is slow, and therefore the shelf life of the product is longer than that of some other fishery products. LAB were not the main bacterial group within the developing spoilage population in vacuum-packaged charcoal-grilled lamprey. There was no association observed between taste scores and APC or LAB

counts. The sensory scores remained almost at the initial level after the storage for 8 weeks and the product was in acceptable condition for at least 5 weeks.

5. *Clostridium botulinum* type E spores were present in the raw river lamprey (1.5%), the suggested count being 100 spores per kg. No growth of C. *perfringens* or *L. monocytogenes* was detected in charcoal-grilled product.The *S. aureus* detected in two samples was probably the result of postprocess contamination.

8 REFERENCES

1. Altschul, S.F., Madden, T.L., Schäffer, A.A., Jinghui Zhang, Zheng Zhang, Miller, W. and Lipman D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25:3389-3402.

2. Amerine, M.A., Pangborn, R.M., Roessler, E.B., 1965. Principles of sensory evaluation of food. Academic Press, New York and London, pp. 354-366.

3. Anonymous. 1964. Botulism outbreak from smoked whitefish. Food Technol. 18:71.

4. Anonymous. 1991a. Elintarvikkeet. Metallipitoisuuksien määrittäminen märkäpolton jälkeen atomiabsorptiospektrofotometrisesti grafiittiuunitekniikalla. Valtion teknillinen tutkimuskeskus. Elintarvikelaboratorio. Menetelmä VTT-4316-91, 7 pp.

5. Anonymous. 1991b. Metaller i svenska havsområden. Swedish Environmental Protection Agency, report 3696, 86 pp.

6. Anonymous. 1968. Fallbericht: Botulismus nach dem Verzehr von geräucherten Lachsforellen. Epidemiologisches Bulletin 4:20.

7. Anonymous. 2003. Results of residue examination of products of animal origin in Finland year 2002. National Food Agency, National Veterinary and Food Research Institute, and Ministry of Agriculture and Forestry, Helsinki, 67 pp.

8. AOAC, 1995. Organochlorine pesticide residues in animal fats, method 984.21. Official Methods of Analysis 16th Ed., Arlington, VA, USA.

9. Aschfalk, A. and Muller, W. 2002. *Clostridium perfringens* toxin types from wild-caught Atlantic cod (*Gadus morhua L.*), determined by PCR and ELISA. Can. J. Microbiol. 48:365-8.

10. Asplund, L., Svensson, B.G., Nilsson, A., Eriksson, U., Jansson, B., Jensen, S., Wideqvist, U. and Skerfving, S. 1994. Polychlorinated biphenyls, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane (p,p'-DDT) and 1,1-dichloro-2,2-bis(p-chlorophenyl)-ethylene (p,p'-DDE) in human plasma related to fish consumption. Arch. Environ. Health 49:477-86.

11. Autio, T., Hielm, S., Miettinen, M., Sjöberg, A-M., Aarnisalo, K., Björkroth, J., Mattila-Sandholm, T. and Korkeala, H. 1999. Sources of *Listeria monocytogenes* contamination in a cold-smoked rainbow trout processing plant detected by pulse-field gel electrophoresis typing. Appl. Environ. Microbiol. 65:150-5.

12. Autio, T.J., Lindström, M.K. and Korkeala, H.J. 2004. Research update on major pathogens associated with fish products and processing of fish. In: Smulders, F.J.M and Collins, J.D. (eds.), Food safety assurance and veterinary public health. Vol 2. Safety assurance during food processing. Wageningen Academic Publishers. Netherlands.

13. Axelsson, L. 2004. Lactic acid bacteria: classification and physiology. In: Salminen, S., von Wright, A. and Ouwehand, A. (eds.), Lactic acid bacteria: microbiology and functional aspects. Marcel Dekker. New York, USA.

14. Ayulo, A.M., Machado, R.A. and Scussel, V.M. 1994. Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. J. Food Prot. 24:171-8.

15. Bagge-Ravn, D., Ng, Y., Hjelm, M., Christiansen, J.N., Johansen, C. and Gram, L. 2003. The microbial ecology of processing equipment in different fish industries-analysis of the microflora during processing and following cleaning and disinfection. Int. J. Food Microbiol. 87:239-50.

16. Bayles, D.O. and Wilkinson, B. 2000. Osmoprotectants and cryoprotectants for *Listeria monocytogenes*. Lett. Appl. Microbiol. 30:23-27.

17. Beaufort, A., Rudelle, S., Gnanou-Besse, N., Toquin, M.T., Bergis, H., Salvat. G. et al. 2004. Prevalence and growth of *Listeria monocytogenes* in cold-smoked salmon.

Proceedings of the XV International Symposium of Problems of Listeriosis; 12-15.9. 2004; Uppsala, Sweden.

18. Bergdoll, M.S. 1989. Enterotoxigenity of staphylococcal strains. In: Doyle, M.P. (ed) Foodborne bacterial pathogens. Marcel Dekker Inc., New York, USA, pp. 479-82.

19. Björkroth, J. and Korkeala, H. 1996a. Evaluation of *Lactobacillus sake* contamination in vacuum packaged sliced cooked meat products by ribotyping. J. Food Prot. 59:398-401.

20. Björkroth, J. and Korkeala, H. 1996b. rRNA gene restriction patterns as a characterization tool for *Lactobacillus sake* strains producing ropy slime. Int. J. Food Microbiol. 30:293-302.

21. Björkroth, J., Vandamme, P. and Korkeala, H. 1998. Identification and characterisation of *Leuconostoc carnosum*, associated with production and spoilage of vacuum-packaged, sliced, cooked ham. Appl. Environ. Microbiol. 64:3313-19.

22. Björkroth, J. and Korkeala, H. 1997. Characterization of *Lactobacillus fructivorans* spoilage in ketchup. J. Food Prot. 60:505-9.

23. Björkroth, K.J., Geisen, R., Schillinger, U., Weiss, N., De Vos, P., Holzapfel, W.H., Korkeala, H.J. and Vandamme, P. 2000. Characterization of *Leuconostoc gasicomitatum* sp. nov. associated with spoiled raw tomato marinated broiler meat strips packaged under modified-atmosphere conditions. Appl. Environ. Microbiol. 66:3764-72.

24. Blumberg, H.M., Kielbauch, J.A. and Wachsmuth, K. 1991. Molecular epidemiology of *Yersinia enterocolitica* O:3 infection: use of chromosomal DNA restriction fragment length polymorphism of rRNA gene. J. Clin. Microbiol. 20:2368-74.

25. Boyer, A., Girault, C., Bauer, F., Korach, J.M., Salomon, J., Moirot, E., Leroy, J. and Bonmarchand, G. 2001. Two cases of foodborne botulism type E and review of epidemiology in France. Eur. J. Clin. Microbiol. Infect. Dis. 20:192-5.

26. Cann, D.C., Wilson, B.B., Hobbs, G., Shewan, J.M. and Johannsen, A. 1965. The incidence of *Clostridium botulinum* type E in fish and bottom deposits in the North Sea and off the coast of Scandinavia. J. Appl. Bacteriol. 28:426-30.

27. Casman, E. P., McCoy, D. W. and Brandly P.J. 1963. Staphylococcal growth and enterotoxin production in meat. Appl. Microbiol. 11:498-500.

28. Civera, T., Parisi, E., Amerio, G.P. and Giaccone, V. 1995. Shelf-life of vacuum-packed smoked salmon; microbiological and chemical changes during storage. Arch. Lebensmittelhyg. 46:1-24.

29. Collins, M.D., Samelis, J., Metaxapoulos, J. and Wallbanks, S. 1993. Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. J. Appl. Bacteriol. 75:595-603.

30. Connor, K.T., Eversen, M., Su, S.H. and Finley, B.L. 2005. Quantitation of polychlorinated biphenyls in fish for human cancer risk assessment: a comparative case study. Environ. Toxicol. Chem. 24:17-24.

31. Dawar, M., Moody, L., Martin, J.D., Fung, C., Isaac-Renton, J. and Patrick, D.M. 2002. Two outbreaks of botulism associated with fermented salmon roe--British Columbia, August 2001. Can. Commun. Dis. Rep. 28:45-9.

32. deBoo, W. 1990. Cadmium in agriculture. Toxicol. Environ. Chem. 27:53-63.

33. Dodds, K.L., Brodsky, H.M. and Warburton, D.W. 1992. A retail survey of smoked ready-to-eat fish to determine their microbiological quality. J. Food Prot. 55:208-210.

34. Dressler, D. 2005. Botulism caused by consumption of smoked salmon. Nervenarzt 76:763-6.

35. EC. 2005. Commission Regulation No 78/2005 amending Regulation No 466/2001 as regards heavy metals.

36. EC. 2006. Commission regulation No 199/2006 Setting maximum levels for certain contaminants in foodstuffs.

37. EC. 2001. Council Regulation No 2375/2001 amending Commission Regulation (EC) No 466/2001 Setting maximum levels for certain contaminants in foodstuffs.

38. Eklund, M. W., Peterson, M. E., Poysky, F. T., Paranjpye, R. N. and Pelroy, G A. 2004. Control of bacterial pathogens during processing of cold-smoked and dried salmon strips. J. Food. Prot. 67:347-51.

39. Falandysz, J. 1985a. Organochlorine pesticides and polychlorinated biphenyls in sprats from the southern Baltic, 1983. Lebensm. Unters. Forsch. 181:482-5.

40. Falandysz, J. 1985b. Trace metals in flatfish from the Southern Baltic, 1983. Lebensm. Unters. Forsch. 181:117-20.

41. Falandysz, J. 1985c. Organochlorine pesticides and polychlorinated biphenyls in flatfish from the southern Baltic, 1983. Lebensm. Unters. Forsch. 181:370-4.

42. Falandysz, J. 1986a. Organochlorine pesticides and polychlorinated biphenyls in herring from the southern Baltic, 1983. Lebensm. Unters. Forsch. 182:131-5.

43. Falandysz, J. 1986b. Trace metals in cod from the southern Baltic, 1983. Lebensm. Unters. Forsch. 182:228-31.

44. Falandysz, J. 1986c. Organochlorine pesticides and polychlorinated biphenyls in cod from the southern Baltic, 1983. Lebensm. Unters. Forsch. 182:136-9.

45. Falandysz, J. 1989. Trace metal levels in the raw and tinned squid Loligo patagonica. Food Addit. Contam. 6:483-8.

46. Falandysz, J., Strandberg, L., Puzyn, T., Gucia, M. and Rappe, C. 2001. Chlorinated cyclodiene pesticide residues in blue mussel, crab, and fish in the Gulf of Gdansk, Baltic Sea. Environ. Sci. Technol. 35:4163-69.

47. Falandysz, J., Wyrzykowska, B., Puzyn, T., Strandberg, L. and Rappe, C. 2002. Polychlorinated biphenyls (PCBs) and their congenerspecific accumulation in edible fish from the Gulf of Gdansk, Baltic Sea. Food. Addit. Contam. 19:779-95.

48. Frimodt, C. 1995. Multilingual illustrated guide to the world's commercial coldwater fish. Fishing News Books, Osney Mead, Oxford, England.

49. Gancel, F., Dzierszinski, F. and Tailliez, R., 1997. Identification and characterization of Lactobacillus spp. isolated from fillets of vacuum-packed smoked and salted herring (*Clupea harengus*). J. Appl. Microbiol. 82, 722-28.

50. Giesy, J.P. and Kannan, K. 1998. Dioxin-like and non-dioxinlike toxic effects of polychlorinated biphenyls (PCBs): implications for risk assessment. Crit. Rev. Toxicol. 28:511-69.

51. Gonzalez-Rodriguez, M. N., Sanz, J.J., Santos, J.A., Otero, A. and Garcia-Lopez, M. L. 2001. Bacteriological quality of aquacultured freshwater fish portions in prepackaged trays stored at 3 degrees C. J. Food. Prot. 64:1399-404.

52. Gonzalez-Rodriguez, M. N., Sanz, J.J., Santos, J.A., Otero, A. and Garcia-Lopez, M. L. 2002. Numbers and types of microorganisms in vacuum-packed cold-smoked freshwater fish at the retail level. 2002. Int. J. Food Microbiol. 77:161-8.

53. Graham, A. F., Mason, D. R., Maxwell, F.J. and Peck, M.W. 1997. Effect of pH and NaCl on growth from spores of non-proteolytic *Clostridium botulinum* at chill temperature. Lett. Appl. Microbiol. 24:95-100.

54. Gram, L. and Huss, H.H. 1996. Microbiological spoilage of fish and fish products. Int. J. Food Microbiol. 33:121-137.

55. Gram, L., Ravn, L., Rasch, M., Bruhn, J.B., Christensen, A.B. and Givskov, M. 2002. Food spoilage – interactions between food spoilage bacteria. Int. J Food Microbiol. 78:79-97.

56. Hallikainen, A., Kiviranta, H., Isosaari, P., Vartiainen, T., Parmanne, R. and Vuorinen, P.J. 2004. Concentrations of dioxins, furans, dioxin-like PCB compounds and polybrominated diphenyl ethers in domestic fresh water and salt water fish. National Food Agency publications 1/2004. Edita Express, Helsinki.

57. Hansen, J.C. and Gilman, A.P. 2005. Exposure of Arctic populations to methylmercury from consumption of marine food: an updated risk-benefit assessment. Int. J. Circumpolar Health 64:301-2.

58. Hardisty, M.W. and Potter, I.C. (eds). 1983. The Biology of Lampreys. Academic Press. London.

59. Hario, M., Himberg, K., Hollmen, T. and Rudbäck, E. 2000. Polychlorinated biphenyls in diseased lesser black-backed gull (*Larus fuscus fuscus*) chicks from the Gulf of Finland. Environ. Pollut. 107:53-60.

60. Hario, M., Hirvi, J.P., Hollmen, T. and Rudbäck, E. 2004. Organochlorine concentrations in diseased vs. healthy gull chicks from the northern Baltic. Environ. Pollut. 127:411-423.

61. Hatakka, M., Björkroth, K.J., Asplund, K., Mäki-Petäys, N. and Korkeala, H. J. 2000. Genotypes and enterotoxicity of *Staphylococcus aureus* isolated from the hands and nasal cavities of flight-catering employees. J. Food Prot. 63:1487-1491.

62. Hatakka, M., Loukaskorpi, M. and Pakkala, P. 2001. Foodborne and waterborne outbreaks in Finland in 2000. National Food Agency Publications No.8/2001, Helsinki, 27 pp.

63. Hauschild, A. H. and Hilsheimer, R. 1977. Enumeration of *Clostridium botulinum* spores in meats by a pour-plate procedure. Can. J. Microbiol. 23:829-832.

64. Hauschild A.H.W. 1989. *Clostridium botulinum*. In: Doyle, M.P. (ed) Foodborne bacterial pathogens. Marcel Dekker Inc., New York, USA, pp. 111-89.

65. HELCOM. 2007. Helsinki Commission. Baltic Marine Environment Protection Comission. http://www.helcom.fi/home/en_GB/welcome/.

66. Hewitt, J.H., Begg, N., Hewish, J., Rawaf, S., Stringer, M. and Theodore-Gandi, B. 1986. Large outbreaks of *Clostridium perfringens* food poisoning associated with the consumption of boiled salmon. J. Hyg. 97:71–80.

67. Hielm S., Hyytiä, E., Andersin, A-B. and Korkeala, H. 1998a. A high prevalence of *Clostridium botulinum* type E in Finnish freshwater and Baltic Sea sediment samples. J. Appl. Microbiol. 84:133-7.

68. Hielm, S., Björkroth, J., Hyytiä, E. and Korkeala, H. 1998b. Prevalence of *Clostridium botulinum* in Finnish trout farms: PFGE typing reveals extensive genetic diversity between type E isolates. Appl. Environ. Microbiol. 64:4161-67.

69. Hielm, S., Björkroth, J., Hyytiä, H. and Korkeala, H. 1998c. Genomic analysis of *Clostridium botulinum* group II by pulsed-field gel electrophoresis. Appl. Environ. Microbiol. 64:703-8.

70. Hielm, S., Lindström, M. and Korkeala, H. 2002. Food safety in seafood; epidemiological concerns related to the geography of fishing grounds. In: Smulders, F.J.M and Collins, J.D. (eds.), Food safety assurance and veterinary public health. Vol 1Food. safety assurance in the pre-harvest phase. Wageningen Academic Publishers. Netherlands.

71. Hirn, J. and Aho, M. 1986. Foodborne disease outbreaks in Finland 1975-1984. Proc. 2nd World Congress Foodborne Infections and Intoxications, Berlin, pp. 94-97.

72. Hoffman, A.D., Gall, K.L., Norton, M. and Wiedmann, M. 2003. *Listeria monocytogenes* contamination patterns for the smoked fish processing environment and for raw fish. Appl. Environ. Microbiol. 66:52-60.

73. Huss, H. H. 1980. Distribution of *Clostridium botulinum*. Appl. Environ. Microbiol. 39:764-9.

74. Huss, H. H. and Pedersen, A. 1979. *Clostridium botulinum* in fish. Nord. Vet. Med. 31:214-21.

75. Hyytiä, E., Hielm, S. and Korkeala, H. 1998. Prevalence of *Clostridium botulinum* type E in Finnish fish and fishery products. Epidemiol. Infect.120:245-50.

76. Hyytiä, E., Hielm, S., Björkroth, J. and Korkeala, H. 1999. Biodiversity of *Clostridium botulinum* type E strains isolated from fish and fishery products. Appl. Environ. Microbiol. 65:2057-64.

77. Hyytiä-Trees, E., Lindström, M., Schalch, B., Stolle, A. and Korkeala, H. 1999. *Clostridium botulinum* type E in Bavarian fish. Arch. Lebensmittelhyg. 50:79-82.

78. Ikonen, E., Kokko U., Tuunainen, P. and Auvinen, H. 1983. Nahkiaisen pyynti Suomessa vuonna 1982. Suomen Kalatalous 50:90-98.

79. International standard, ISO 4833, 1991. Microbiology – General guidance for the enumeration of micro-organisms - Colony count technique at 30° C. 2nd ed. International Organization for Standardization. Geneva, 5 pp.

80. Janvier, P. 1997. Tree of life. http://tolweb.org/tree?group=Hyperoartia&contgroup=Vertebrata.

81. Jeppesen, V.T. and Huss, H.H. 1993. Characteristic and antagonistic activity of lactic acid bacteria isolated from chilled fish products, Int. J. Food Microbiol. 18:305-20.

82. Johannsen, A. 1962. Förekomst och utbredning av *Cl. botulinum* typ E med särskilt hänsyn till Öresundområdet. Nord. Vet. Med. 14:441-74.

83. Johannsen, A. 1963. *Clostridium botulinum* in Sweden and the adjacent waters. J. Appl. Bacteriol. 91:964-74.

84. Johansson, T., Rantala, L., Palmu, L. and Honkanen-Buzalski, T. 1999. Occurrence and typing of *Listeria monocytogenes* strains in retail vacuum-packed fish product and in a production plant. Int. J. Food Microbiol. 47:111-9.

85. Junttila, J. R., Niemelä, S.I. and Hirn, J. 1988. Minimum growth temperatures of *Listeria monocytogenes* and non-haemolytic listeria. J. Appl. Bacteriol. 65:321-27.

86. Järvenpää, T. and Westman, K. 1986. Nahkiainen-nejonögon-symposium 17.-18.10.1979 Kalajoki. Kalastusmuseoyhdistyksen julkaisuja - fiskerimuseiföreningens publikationer. Kalastusmuseoyhdistys. Helsinki, 32 pp.

87. Kainua, K. and Valtonen, T. 1980 Distribution and abundance of European river lamprey (*Lampetra fluviatilis*) larva in three rivers running into Bothnian Bay, Finland. Can. J. Fish. Aquat. Sci. 37:1960-1966.

88. Karl, H. and Lehmann, I. 1993. Organochlorine residues in edible parts of eels of different origin. Lebensm. Unters. Forsch. 197:385-388.

89. Karnop, G. 1980. Qualität und Lagerverhalten heiss geräucherter Fischereiprodukte. I. Sensorische und mikrobiologische Beschaffenheit von rauchfrischem Heilbutt, Bückling und Aal. Deutsche Lebensm. Rundschau 76:42–47.

90. Kiviranta, H., Vartiainen, T., Parmanne, R., Hallikainen, A. and Koistinen, J. 2003. PCDD/Fs and PCBs in Baltic herring during the 1990s. Chemosphere 50:1201-16.

91. Kleickmann, A. and Schellhaas, G. 1979. Zum Keimgehalt von Räucherfischen. Arch. Lebensmittelhyg. 30:26-29.

92. Korkeala H., and Pakkala, P. 1988. Microbiological changes in smoked and charred Baltic herrings during storage. J. Food Prot. 51:197-200.

93. Korkeala, H., Stengel, G., Hyytiä, E., Vogelsang, B.,Bohl, A.,Wihlman, H., Pakkala, P. and Hielm, S. 1998. Type E botulism associated with vacuum-packaged hot-smoked whitefish. Int. J. Food Microbiol. 43:1-5.

94. Kukko, O. and Turunen, J. 1973. Perämeren jokien nahkiaisen, *Lampetra fluviatilis* elohopeapitoisuudesta. Ympäristö ja terveys 3:298-302.

95. Labbe, R.G. and Juneja, V.K. 2002. *Clostridium perfringens,* in: Cliver, D.O. and Riemann, H.P. (ed.) Foodborne Diseases, 2nd Edition. Academic Press. Amsterdam.

96. Lappi, V.R., Ho, A., Gall, K. and Wiedmann, M. 2004. Prevalence and growth of Listeria on naturally contaminated smoked salmon over 28 days of storage at 4 degrees C. J. Food Prot. 67:1022-6.

97. Laukkanen, A., Mikkonen, A., Laaksonen, P. and Saure, L. 1979. Physiological features of adult river lamprey (*Lampetra fluviatilis*) during wintering in Northern Finland. Poster lecture. Sea lamprey int. symp. Marquette. Michigan. USA.

98. Lee, N.K., Jun, S.A., Ha, J.U. and Paik, H.-D. 2000. Screening and characterization of bacteriocinogenic lactic acid bacteria from jeot-gal, a Korean fermented fish food. J. Microbiol. Biotechnol. 10:423-8.

99. Leivuori, M. and Niemistö L. 1993. Trace metals in the sediment of the Gulf of Bothnia. Aqua Fennica 23:89-100.

100. Leroi, F., Joffraud, J., Chevalier, F. and Cardinal, M. 1998. Study of the microbiological ecology of cold-smoked salmon during storage at 8°C. Int. J. Food Microbiol. 39:111-21.

101. Lina, G., Boutite, F., Tristan, A., Bes, M., Etienne, J. and Vandenesch, F. 2003. Bacterial competition for human nasal cavity colonization: role of staphylococcal *agr* alleles. Appl. Environ. Microbiol. 69:18-23.

102. Lindsjö, O. and Riekkola M., 1976. Atomiabsorptiospektrofotometria. Instrumenttianalytiikka, 178.

103. Lindström, M., Hielm, S., Nevas, M., Tuisku, S. and Korkeala, H. 2004. Proteolytic *Clostridium botulinum* type B in the gastric content of a patient with type E botulism due to whitefish eggs. Foodborne Pathogens Dis. 1:53-8.

104. Lindström, M., Keto, R., Markkula, A., Nevas, M., Hjelm, S. and Korkeala, H. 2001. Multiplex PCR assay for detection and identification of *Clostridium botulinum* types A, B, E and F in food and fecal material. Appl. Environ. Microbiol. 67:5694-99.

105. Lindström, M. and Korkeala, H. 2006. Laboratory diagnostic of botulism. Clin. Microbiol. Rew. 19:298-314.

106. Lindström, M., Nevas, M., Hielm, S., Lähteenmäki, L., Peck, M.W. and Korkeala, H. 2003. Thermal inactivation of nonproteolytic *Clostridium botulinum* type E spores in model fish media and in vacuum-packaged hot-smoked fish products. Appl. Environ. Microbiol. 69:4029-36.

107. Lindström, M., Vuorela, M., Hinderink, K., Korkeala, H.,Dahlsten,E., Raahenmaa, M and Kuusi, M. 2006. Botulism associated with vacuum-packed smoked whitefish in Finland, June-July 2006. Eurosurveillance weekly report. 11:7. www.eurosurveillance.org.

108. Linnan, M.J., Mascola, L., Lou, X.D., Goulet, V., May, S., Salminen, C., Hird, D.W., Yonekura, M.L., Hayes, P.and Weaver, R. 1988. Epidemic listeriosis associated with Mexican-style cheese. N. Engl. J. Med. 319:823-8.

109. Lundén, J. 2004. Persistent *Listeria monocytogenes* contamination in food processing plants. Academic Dissertation. University of Helsinki. Helsinki, 60 pp.

110. Lyhs, U. 2002. Lactic acid bacteria associated with the spoilage of fish products. Academic dissertation. University of Helsinki. Helsinki, 77 pp.

111. Lyhs, U., Björkroth, J., Hyytiä E. and Korkeala H. 1998. The spoilage flora of vacuum-packaged, sodium nitrite or potassium nitrite treated, cold-smoked rainbow trout stored at 4° C or 8° C. Int. J. Food Microbiol. 45:135-142.

112. Lyhs, U., Björkroth, J. and Korkeala H. 1999. Characterisation of lactic acid bacteria from spoiled, vacuum-packaged, cold-smoked rainbow trout using ribotyping. Int. J. Food Microbiol. 52:77-84.

113. Lyhs, U., Korkeala, H., Vandamme, P. and Björkroth, J. 2001. *Lactobacillus alimentarius*: a specific spoilage organism in marinated herring. Int. J. Food Microbiol. 60:355-60.

114. Mackenzie, B.R., Almesjo, L. and Hansson, S. 2004. Fish, fishing, and pollutant reduction in the Baltic Sea. Environ. Sci. Technol. 38:1970-76.

115. Magnússon, H. and Traustadóttir, K. 1982. The microbial flora of vacuum-packed smoked herring fillets. J. Food Technol. 17:695-702.

116. McClane, B.A., Hanna, P.C. and Wnek, A.P. 1988. *Clostridium perfringens* enterotoxin. Microb. Pathog. 5:317-23.

117. McClure, P.J., Kelly, T.M., and Roberts, T.A. 1991. The effects on temperature, pH, sodium chloride and sodium nitrite on the growth of *Listeria monocytogenes*. Int. J. Food Microbiol. 14:77-91.

118. Merivirta, L. 2006. Paistettuja nahkiaisia ja silmusoppaa. Suomen Eläinlääkärilehti 10:536-7.

119. Miettinen, H. and Wirtanen, G. 2005. Prevalence and location of *Listeria monocytogenes* in farmed rainbow trout. Int. J. Food Microbiol. 104:135-43.

120. Miettinen, M.K., Siitonen, A., Heiskanen, P., Haajanen, H., Björkroth, K.J. and Korkeala, H. 1999. Molecular epidemiology of an outbreak of febrile gastroenteritis caused by *Listeria monocytogenes* in cold-smoked rainbow trout. J. Clin. Microbiol. 37:2358-60.

121. Ministry of Agriculture and Forestry, Information Center. 2003. Yearbook of Farm Statistic, Helsinki, 398 pp.

122. Nakari, T., Nuutinen, J., Pehkonen, R. and Järvinen, O. 2004. Uppföljning av miljögifter i vattendrag och kustvatten 2000-2002. Finlands miljöcentral. Helsingfors, 35 pp.

123. Nakari, T., Suortti, A-M. and Järvinen, O. 2002. Monitoring of toxic compounds in fresh and coastal waters in 1997-1999. Finnish Environment Institute. Helsinki, 64 pp.

124. NCFA, 1984. Measuring of water activity with a polyamide filament hygrometer. Nordic Committee on Food Analysis Nr, 104, 3 pp.

125. NCFA, 1990. *Listeria monocytogenes*. Detection in foods. Nordic Committee on Food Analysis Nr, 136, 9 pp.

126. NCFA, 1991. Lactic acid bacteria. Determination in meat and meat products. Nordic Committee on Food Analysis Nr, 140, 4 pp.

127. NCFA, 1992a. *Enterococcus*. Determination in foods. Nordic Committee on Food analysis Nr, 68, 2nd ed., 6 pp.

128. NCFA, 1992b. *Staphylococcus aureus*. Determination in foods. Nordic Committee on Food Analysis Nr, 66, 2nd ed., 6 pp.

129. NCFA, 1997. *Clostridium perfringens*. Determination in foods. Nordic Committee on Food Analysis Nr, 95, 3rd ed., 4 pp.

130. Nelson, J.S. 1994. Fishes of the world. Third edition. John Wiley & Sons, Inc., New York, 600 pp.

131. Novak, J.S., Peck, M.W., Juneja, V.K. and Johnson, E.A. 2005. *Clostridium botulinum* and *Clostridium perfringens*. In: Fratamico, P.M., Bhunia, A.K. and Smith, J.L. (ed.) Foodborne Pathogens, Microbiology and Molecular Biology. Caister Academic Press, Norfolk, UK, pp. 383-407.

132. Official Statistic of Finland, 1999. Fish Processing in 1997. Finnish Game and Fisheries Research Institute. Helsinki, Finland, pp. 16-17.

133. Olsson, A., Vitinsh, M., Plikshs, M. and Bergman, A. 1999. Halogenated environmental contaminants in perch (*Perca fluviatilis*) from Latvian coastal areas. Sci. Tot. Environ. 239:19-30.

134. Paleari, M.A., Soncini, G. and Beretta, G. 1989. Smoked tuna, sliced and vacuum packed, a relatively new product. Lebensm. Unters. Forsch. 190:118-120.

135. Paludan-Müller, C., Dalgaard, P., Huss, H.H. and Gram, L. 1998. Evaluation of the role of Carnobacterium piscicola in spoilage of vacuum- and modified-atmosphere-packed cold-smoked salmon stored at 5 degrees C. Int. J. Food Microbiol. 39:155-66.

136. Paludan-Müller, C., Huss, H.H., and Gram, L. 1999. Characterization of lactic acid bacteria isolated from a Thai low-salt fermented fish product and the role of garlic as substrate for fermentation. Int. J. Food Microbiol. 46:193-200.

137. Paoli, G.C., Bhunia, A.K. and Bayles, D.O. 2005. *Listeria monocytogenes.* In: Fratamico, P.M., Bhunia, A.K. and Smith, J.L. (ed.) Foodborne Pathogens, Microbiology and Molecular Biology. Caister Academic Press, Norfolk, UK, pp. 273-293.

138. Pitcher, D.G., Saunders, N.A. and Owen, R.J., 1989. Rapid extraction of bacteria genomic DNA with guanidium thiocyanate. Lett. Appl. Microbiol. 8:151-156.

139. Raitaniemi, J., Manninen, K. and Stigzelius, J. (Eds.) 2004. Kalavarat 2004. Finnish Game and Fisheries Research Institute. Helsinki, Finland, pp. 55-56.

140. Ringø, E. 2004. Lactic acid bacteria in fish and fish farming. In: Salminen, S., von Wright, A. and Ouwehand, A. (eds.), Lactic-acid-bacteria:-microbiology-and-functional-aspects. Marcel Dekker. New York, USA.

141. Ringø, E., Bendiksen, H.R., Wesmanjervi, M.S., Olsen, R.E., Jansen, P.A. and Mikkelsen, H. 2000. Lactic acid bacteria associated with the digestive tract of Atlantic salmon (*Salmo salar L.*). J. Appl. Microbiol. 89:317:22.

142. Ringø, E., Wesmanjervi, M.S., Bendiksen, H.R., Berg, A., Olsen, R.E., Johnsen, T., Mikkelsen, H., Seppola, M., Strom, E. and Holzapfel, W. 2001. Indentification of Carnobacteria isolated from fish intestine. Syst. Appl. Microbiol. 24:183-91.

143. Roots, O. 2001. Halogenated environmental contaminants in fish from Estonian coastal areas. Chemosphere 43:623-32.

144. Routti, H., Nyman, M., Backman, C., Koistinen, J. and Helle, E. 2005. Accumulation of dietary organochlorines and vitamins in Baltic seals. Mar. Environ. Res. 60:267-287.

145. Schlech, W.F.3rd, Lavigne, P.M., Bortolussi, A.C., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V. 1983. Epidemic listeriosis: Evidence for transmission by food. N. J. Engl. Med. 308:203-6.

146. Seeliger, H.P.R. and Hohne, K. 1979. Serotyping of *Listeria monocytogenes* and realted species. Meth. Microbiol. 13:31-49.

147. Shimasaki, T., Miake, K., Tsukamasa, Y., Sugiyama, M., Minegishi, Y. and Shinano, H., 1994. Effect of water activity and storage temperature on the quality and microflora of smoked salmon. Nippon Suisan Gakkaishi 60:569-576.

148. Sikorski, Z. and Kolodziejska, I. 2002. Microbial risks in mild hot smoking of fish. Crit. Rev. Food Sci. 42:35-51.

149. Smith, L. D. S. and Sugiyama, H. 1988. Cultural and serological characteristics. In: Smith, L. D. S. and Sugiyama, H. (eds.) Botulism: The organism, its toxins, the disease. Charles C. Thomas, Springfield, USA, pp. 23-37.

150. Soimasuo, M., Kervinen, J., Sinkkonen, S. and Paasivirta, J. 2004. Bioaccumulation of POPs from Contaminated Sediment to Lamprey(*Lampetra fluviatilis* L.) Larva. J. Soil & Sediments. 4:75-83.

151. Specht, W. 1987. Method 6 and S 19. Manual of Pesticide Residue Analysis, Vol 1, ed. by Deutsche Forschungsgemeinschaft, Weinheim.

152. Stewart, G.C. 2005. *Staphylococcus aureus* In: Fratamico, P.M., Bhunia, A.K. and Smith, J.L. (eds.) Foodborne Pathogens, Microbiology and molecular biology. Caister Academic Press, Norfolk, UK, pp. 273-284.

153. Strandberg, B., Strandberg, L., van Bavel, B., Bergqvist, P.A., Broman, D., Falandysz, J., Näf, C., Papakosta, O., Rolff, C. and Rappe, C. 1998a. Concentrations and spatial variations of cyclodienes and other organochlorines in herring and perch from the Baltic Sea. Sci. Tot. Environ. 215:69-83.

154. Strandberg, B., Bandh, C., van Bavel, B., Bergqvist, P.A., Broman, D., Ishaq, R., Naf. C. and Rappe, C. 2000. Organochlorine compounds in the Gulf of Bothnia: sediment and benthic species. Chemosphere 40:1205-1211.

155. Strandberg, B., Bandh, C., van Bavel, B., Bergqvist, P.A., Broman, D., Naf, C., Pettersen, H. and Rappe, C. 1998b. Concentrations, biomagnification and spatial variation of organochlorine compounds in a pelagic food web in the northern part of the Baltic Sea. Sci. Tot. Environ. 217:143-154.

156. Szefer, P. and Falandysz, J. 1985. Trace metals in muscle tissue of fish taken from the Southern Baltic. Lebensm. Unters. Forsch. 181:217-220.

157. Tanasupawat, S., Shida, O., Okada, S. and Komagata, K. 2000. *Lactobacillus acidopiscis* sp. nov. and *Weissella thailandensis* sp. nov., isolated from fermented fish in Thailand. Int. J. Syst. Evol. Microbiol. 50:1479-1485.

158. Thimothe, J., Nightingale, K.K., Gall, K., Scott, V.N. and Wiedmann, M. 2004. Tracking of *Listeria monocytogenes* in smoked fish processing plants. J. Food Prot. 67:328-41.

159. Thomas, H. A. 1942. Bacterial densities from fermentation tube tests. J. Am. Water Works Assoc. 34:572-6.

160. Truelstrup Hansen, L. 1995. Quality of chilled, vacuum-packed cold-smoked salmon. Thesis, Danish Institute of Fisheries Research, Department of Seafood Research, Technical University, Denmark.

161. Tuikkala, A. 1971. Nahkiaisen elintavoista ja sen pyynnistä Pyhäjoella. Kalataloussäät. Monist. Julk. 40:1-59.

162. Tuunainen, P., Ikonen, E. and Auvinen. H. 1980. Lamprey and lamprey fisheries in Finland. Can. J. Fish. Aquat. Sci. 37:1953-59.

163. van den Berg, M., Birnbaum, L., Bosveld, A.T., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., van Leeuwen, F.X., Liem, A.K., Nolt, K., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillit, D., Tysklind, M., Younes, M., Waern, F. and Zacharewski, T. 1998. Toxic equvalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Persp. 106:775-92.

164. Walker, S.J., Archer, P. and Banks, J.G. 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. 68:157-62.

165. Welz, B. 1983. Atomabsorptionspektrometrie, Weinheim, Verlag Chemie GmbH, 321 pp.

166. Venäläinen, E-R., Hallikainen, A., Parmanne, R. and Vuorinen, P.J. 2004. Heavy metal contents in Finnish sea and fresh water fish. National Food Agency 3/2004. Helsinki.

167. Wieneke, A.A., Roberts, D. and Gilbert, R.J. 1993. Staphylococcal food poisoning in the United Kingdom 1969-90. Epidemiol. Infect. 110:519-31.

168. von Zorn, W., Greuel, E. and Krämer, J. 1993. Beurteilung des Hygienestatus geräucherter, vakuumverpackter Forellenfilets. Arch. Lebensmittelhyg. 44:95-8.